American Academy of Allergy, Eighth Annual Meeting, Hotel Sherman, Chicago, Ill., Feb. 18 to 20, 1952.

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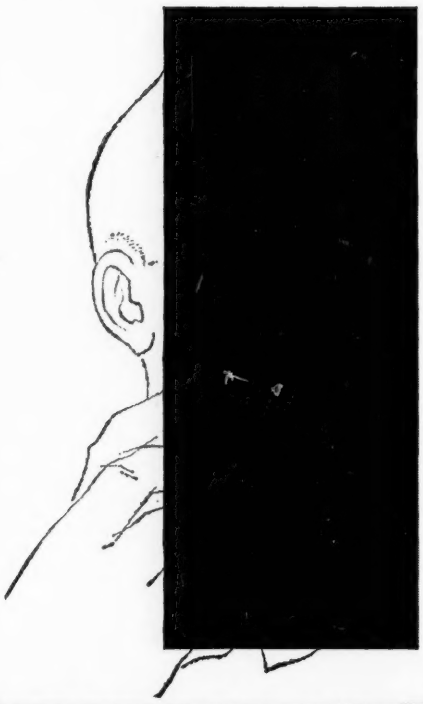

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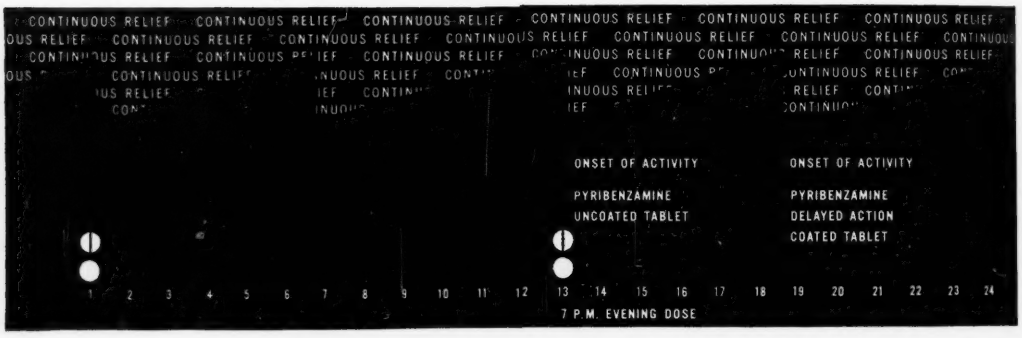
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The Journal of Allergy

VOL. 22 JULY, 1951 NO. 4

Original Articles

ORAL CORTISONE THERAPY IN ALLERGIC DISEASES*†

SIDNEY FRIEDLAENDER, M.D., AND ALEX S. FRIEDLAENDER, M.D.
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THE favorable influence of ACTH and parenterally administered cortisone on diseases of hypersensitivity has been noted in a number of reports during the past year.[1-3] In many instances of allergic disease, prompt and complete remissions lasting for variable periods of time have occurred following a single course of treatment with these hormones. The tendency of allergic symptoms to be influenced by many specific and nonspecific factors must of course be taken into consideration in assessing the effect of any therapeutic agent. There appears to be little doubt, however, in the opinions of many who have used these agents in a controlled manner, that ACTH and cortisone are frequently capable of interrupting or alleviating, temporarily at least, allergic type reactions of a severe nature.

The present report deals with the effects of orally administered cortisone acetate in allergic disease. The preparation of cortisone which was supplied for this study in the form of 25 mg. tablets‡ was first used in cases of ragweed pollinosis during the latter part of the 1950 pollinating season. The improvement which occurred in a number of patients immediately following its use, together with a depressing effect on the blood eosinophile level in many instances, indicated that the orally administered hormone was readily absorbed from the gastrointestinal tract. Its use was extended to patients suffering from asthma, atopic and contact dermatitis, severe urticaria, and angioneurotic edema. Efforts were made to evaluate objectively the clinical response and to determine optimum dosage requirements. Where possible the relative effectiveness of oral and injectable cortisone was compared in the same patient. The subjects used in the study were ambulatory, and were seen at frequent intervals, daily when possible, in order to record subjective and objective signs of improvements closely, and to follow blood eosinophile levels, body weights, and blood pressures. It was also felt that frequent observation was essential to control any of the untoward effects known to occur from adrenal cortical hormone therapy.

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†Presented at the Seventh Annual meeting, American Academy of Allergy, New York, N. Y., Feb. 7, 1951.

‡Oral cortisone acetate (Cortone-Merck) was made available through the courtesy of Dr. J. M. Carlisle, Medical Director, Merck & Company, Inc., Rahway, N. J.

Effect on Blood Eosinophiles.—The fall in the level of blood eosinophiles has been shown to be a relatively simple measure of increased adrenal cortical function. In order to help determine the degree and rapidity of absorption of oral cortisone from the gastrointestinal tract, total blood eosinophiles were determined by a method described by Pilot,[4] a modification of Randolph's technique, which in our experience allows a rapid and accurate determination of such cells in the blood. In one group of 8 subjects who presented an eosinophilia of 5 per cent or greater, serial determinations of the total blood eosinophiles were made during a 4 hour period after a single test dose of 25 or 50 mg. of oral cortisone (Table I). A drop in total eosinophiles ranging from 22 to 60 per cent occurred at the end of 4 hours in 4 subjects who received a single dose of 25 mg. In those who were given 50 mg., a decrease of 31 to 44 per cent occurred in 3 subjects, while an increase of 25 per cent was recorded in the fourth.

TABLE I. THE EFFECT OF A SINGLE DOSE OF ORAL CORTISONE ON BLOOD EOSINOPHILE LEVELS

CASE NUMBER	RESTING EOSINOPHILES		DOSE OF CORTISONE (MG.)	TOTAL EOSINOPHILES PER C.MM. AT					PER CENT CHANGE
	PER CENT	TOTAL PER C.MM.		½ HR.	1 HR.	2 HR.	3 HR.	4 HR.	
1	9	566	25	433	494	389	250	239	–60
2	17	1676	25	1643	1648	1420	1399	1265	–25
3	5	133	25	106	72	178	105	94	–29
4	21	2042	25		1876	2735	1832	1610	–22
5	17	2364	50		2664	2220	1610	1343	–44
6	13	512	50		544	512	427	355	–31
7	11	622	50		577	450	444	355	–43
8	11	866	50		905	971	1098	1088	+25

TABLE II. EFFECT OF ORAL CORTISONE THERAPY ON BLOOD EOSINOPHILE LEVELS

CASE NUMBER	EOSINOPHILES PER C.MM. OF BLOOD		PER CENT FALL	DAY OF MAXIMUM FALL	THERAPEUTIC EFFECT
	BEFORE TREATMENT	MAXIMUM FALL DURING TREATMENT			
1	776	111	–85	2	good
2	1631	277	–90	4	good
3	233	33	–86	2	fair
4	544	111	–80	3	good
5	866	56	–94	4	good
6	1798	200	–89	2	fair
7	1276	255	–80	5	good
8	1809	45	–98	4	good
9	278	244	–10	2	good
10	777	122	–85	3	good
11	598	178	–70	2	good
12	2331	300	–87	3	good
13	222	increase	–	–	poor
14	422	78	–82	4	good
15	577	66	–89	2	good
16	1176	178	–85	3	poor
17	255	66	–74	3	poor
18	1365	932	–32	2	poor
19	1132	44	–96	2	poor
20	577	67	–89	2	fair
21	900	200	–78	2	good
22	344	55	–84	2	good

In patients who were treated with oral cortisone at intervals of 4, 6, or 8 hours over a period of days, the depression in eosinophiles was more marked at the end of 24 hours, and reached a maximum depression from the second to fifth day of treatment (Table II). When the dose of cortisone was reduced, the eosinophile level usually rose, though rarely attaining the higher initial levels. In those who were symptomatically benefited, such improvement often persisted for some period after complete withdrawal of the hormone, despite an increase in eosinophiles.

While the degree of clinical improvement usually paralleled the eosinopenia, a poor clinical effect in some instances occurred in patients showing a marked eosinophile depression. In relatively few cases, however, did clinical improvement occur in the absence of a depressing effect on these cells. In one instance the opportunity presented itself to use the injectable form of cortisone in a patient who did not respond favorably while taking the oral material, and in whom eosinophiles were not reduced. An equivalent amount of injectable cortisone produced a clinical remission and an eosinopenia, sug-

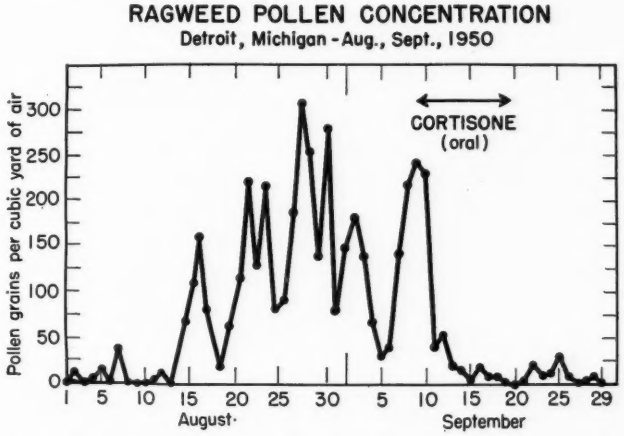

Fig. 1.—Period of oral cortisone administration in ragweed hay fever patients.

gesting that in this instance, at least, orally administered cortisone was poorly absorbed. However, the evidence gathered in the majority of the cases included in this study indicates that oral cortisone is ordinarily absorbed quite rapidly and completely from the gastrointestinal tract.

Effect on Ragweed Hay Fever.—Eight patients with severe ragweed hay fever received oral cortisone late in the 1950 pollinating season. Ragweed pollen sensitivity was the primary consideration in each of these subjects. The onset of symptoms was coincident with the appearance of ragweed pollen in the air, and cutaneous reaction to ragweed pollen extract was strong in each instance. These patients had received little or no prior hyposensitization therapy. Antihistaminic drugs and other symptomatic medicaments were not alleviating symptoms at the time hormone therapy was begun. Unfortunately, the oral cortisone was received late in the season, and administration was started at a period of high pollen concentration which was followed in 48

hours by a rapid fall (Fig. 1). The pollen concentration then remained low during the balance of the season. According to the pollen record, the pollen count on September 9, the day on which treatment was begun in each patient, was 237 gr. per cubic yard of air.

The initial dosage was 25 mg. every 4 hours. A remarkable improvement occurred in 6 of the 8 hay fever sufferers during the first 24 hours of oral cortisone treatment. In those who were benefited during this period, the onset of improvement was reported 4 to 8 hours after the first dose was taken. Rhinorrhea, sneezing, itching of the eyes and nose, and nasal blocking appeared to be equally relieved. After the first 24 hours, when symptoms appeared to be definitely relieved, the dose was reduced to 25 mg. every 6 hours for 3 additional days, then progressively reduced and discontinued on the tenth day of treatment. Symptoms did not recur during the balance of the season in these 6 patients. It was felt that symptoms in these patients were influenced by the hormone, although the cortisone could hardly be credited for the continued remission since the pollen concentration remained low for the balance of the season. Such a prompt and dramatic improvement failed to occur during this period in 2 other patients treated with oral cortisone and in a number of control subjects.

Two subjects who failed to improve during cortisone therapy received the hormone for a total of 6 days. Both showed a depression in the eosinophile level during treatment. One had previously failed to improve during a 10-day period from August 21 to 31 when he received a daily injection of 100 mg. of cortisone suspension. The symptoms started to subside on September 16, 3 days after oral cortisone was discontinued. The second patient who did not improve during therapy subsequently developed asthma and a secondary bronchial infection, and was treated with conventional symptomatic measures and antibiotics. All symptoms in this case disappeared by mid-October.

Effect on Bronchial Asthma.—Seventeen ambulatory patients with severe asthma were given oral cortisone for a sufficient period of time to make an evaluation of its effect in each instance (Table III). The only criterion used in the selection of these cases for study was the presence of daily asthmatic symptoms for one month or more, requiring constant symptomatic measures which were insufficient to control their difficulty adequately. The patients ranged in age from 12 to 62 years, with histories of asthma dating back 2 to 35 years. Allergic investigation in the majority of these patients revealed sensitivities to various of the common seasonal and nonseasonal inhalants and to food allergens. In some instances, specific extrinsic sensitizations could not be determined. The history in each case indicated that symptoms were not limited to any particular season, although the fall of the year, which was the period of this study, was usually a bad period for each of them. Twelve subjects were considered not to have had adequate allergic management up to this time. Five others were under care for one year or longer and were still manifesting recurrent episodes of asthma. Each patient was being seen frequently at regular intervals before cortisone therapy, and in order to minimize

as much as possible the psychologic influences associated with the introduction of new types of treatment, the nature of the oral hormone therapy was not discussed with them. Symptomatic measures such as aminophylline, epinephrine, ephedrine, and iodides were already in use, and were continued as required during the period of cortisone treatment. The requirements for such medication served as a helpful guide in evaluating the extent of improvement occurring during treatment. Placebo therapy was frequently utilized to confirm the impression that the clinical effects observed were due to the hormone.

The initial daily dose in all patients was 100 to 200 mg. In all but one instance, where the dose was later increased to 300 mg., the initial daily dose represented the maximum administered during the course of treatment. This was given in amounts of 25 or 50 mg. at intervals of 4, 6, or 8 hours, and continued for a long enough period to determine clinical effect, and then progressively reduced over a period of days before being discontinued. Toward the end of each course, the intervals between doses were increased to 12 and finally to 24 hours. The periods of hormone administration ranged from 6 to 18 days, with an average of 10 days. The total dosage administered varied from 500 to 1,700 mg. with an average of 1,000 mg. for each course.

The clinical response during cortisone therapy was considered good in 12 cases, fair in 2, and poor in 3 others. In those who improved during the period of treatment, the initial subjective improvement was frequently reported within the first 6 hours, reaching a maximum after 24 to 48 hours of therapy. Subjective improvement reported by the patient usually consisted of relief of heaviness in the chest, reduction or disappearance of cough and wheezing, a general feeling of well-being, and the ability to relax and sleep for longer intervals than had been possible for some time. Where large amounts of sputum were present, the quantity of the secretion appeared to diminish, although its character did not change. If the sputum was purulent prior to therapy, it usually remained so, and in a number of instances the sputum, which had previously been clear, assumed a purulent character during the period of cortisone administration. Objective signs of improvement consisted of disappearance of the auscultatory findings of asthma together with an increase in vital capacity. In those who were considered to have attained a good response during cortisone therapy, the objective as well as subjective signs of asthma completely disappeared.

The complete remissions which occurred during cortisone therapy were generally of short duration. In one patient, asthma recurred on the fourth day of therapy when the daily dose was reduced to 75 mg., and in 2 others it recurred on the third and fifth day when the dose was reduced to 50 mg. Symptoms in each case disappeared when the higher initial dosage was restored for a period of 24 hours. Asthma did not recur when the dose was again reduced, but symptoms appeared 3 to 5 days after cortisone was discontinued. In the remaining 9 patients who improved during cortisone therapy, complete freedom lasted for periods ranging from 1 to 11 days in 8 patients, and in one patient for as long as 35 days after cortisone was discontinued. The severity of the asthma which eventually recurred was, in most

TABLE III. EFFECT OF ORAL CORTISONE IN BRONCHIAL ASTHMA

CASE NUMBER	AGE (YEARS)	SEX	DURATION OF SYMPTOMS (YEARS)	ETIOLOGIC FACTORS	CORTISONE			THERAPEUTIC EFFECT			VITAL CAPACITY		LENGTH OF REMISSION AFTER CORTISONE DISCONTINUED (DAYS)
					TOTAL DOSE (MG.)	MAXIMUM DAILY DOSE (MG.)	DAYS THERAPY	DEGREE	INITIALLY NOTED (HOURS)	MAXIMUM OBTAINED (HOURS)	BEFORE THERAPY (C.C.)	MAXIMUM INCREASE (C.C.)	
1	48	F	35	E & I	1050	150	11	good	12	24	800	2800	3
2	12	M	8	E	500	100	7	good	4	24	1000	1800	35
3	40	M	18	E & I	825	150	6	fair	12	24	2600	3400	8
4	35	F	30	E & I	600	200	9	good	6	72	1200	1800	4
5	48	F	32	E & I	775	150	8	good	24	48	1200	200	2
6	50	F	20	E & I	800	200	8	fair	2	48	600	1400	5
7	53	M	7	E & I	1150	200	15	good	24	72	1400	4000	2
8	13	F	2	E	1300	200	13	good	6	24	1200	3000	11
9	18	F	8	E	975	200	8	good	8	24	1600	3000	4
10	62	M	5	I	1300	200	18	good	3	48	1600	3600	4
11	39	F	3	E & I	1000	200	14	good	6	24	1000	2800	8
12	31	F	8	E	1125	200	10	good	24	72	1000	2400	4
13	55	M	15	E & I	900	200	6	poor	–	–	1100	–	–
14	52	M	11	E & I	1025	150	11	good	6	48	2000	3200	3
15	56	M	12	I	825	150	8	good	6	48	1800	3200	1
16	24	F	16	E & I	1100	200	11	poor	–	–	1200	–	–
17	36	F	10	E & I	1700	300	13	poor	–	–	1400	–	–

E — extrinsic.
I — intrinsic.

cases, equivalent to or greater than that which existed prior to hormone treatment. In 6 patients who were given a second course of cortisone following recurrence of symptoms, the pattern of response was similar to that noted during the initial course (Table IV). Asthma again recurred shortly after cortisone was discontinued in all cases.

TABLE IV. COMPARATIVE EFFECT OF FIRST AND SECOND COURSES OF ORAL CORTISONE IN ASTHMA

CASE NUMBER	COURSE NUMBER	INTERVAL BETWEEN COURSES (DAYS)	CORTISONE			THERAPEUTIC EFFECT	LENGTH OF REMISSION AFTER CORTISONE DISCONTINUED (DAYS)	SEVERITY AFTER RELAPSE
			TOTAL DOSE (MG.)	MAXIMUM DAILY DOSE (MG.)	DAYS OF TREATMENT			
2	1	60	500	100	7	good	35	severe
	2		700	100	14	good	21	severe
7	1	3	1150	200	15	good	2	severe
	2		1000	200	8	good	2	severe
9	1	5	975	200	8	good	4	severe
	2		1050	200	14	good	14	moderate
10	1	6	1300	200	18	good	4	severe
	2		1100	200	13	good	3	severe
12	1	12	1125	200	10	good	4	severe
	2		1100	200	14	good	7	moderate
15	1	20	825	150	8	good	1	moderate
	2		950	150	12	good	3	moderate

Of those who failed to respond favorably during cortisone treatment, one was given 200 mg. daily for 5 days, and in the absence of any noticeable improvement, the dose was increased to 300 mg. for 2 additional days. Thereafter the dosage was reduced over a period of days and discontinued. This patient received a total of 1,700 mg. of cortisone, the largest amount of any included in this series. Despite no improvement in this patient's condition, a 74 per cent fall in blood eosinophiles was recorded. A second patient who received oral cortisone without clinical improvement or reduction in eosinophiles had previously improved after a course of ACTH. Following his failure to improve on oral cortisone, he was hospitalized and given a course of the cortisone suspension by injection. He attained a remission which was credited to the influence of the hormone, and at that time also showed a depression in the blood eosinophile level. As previously pointed out, it would appear in this case that there was some impairment in the absorption of the orally administered cortisone. A third patient was not benefited by oral cortisone despite an 85 per cent fall in the eosinophile level. Two patients were improved during cortisone therapy, but were never completely free of asthma. Their eosinophile response during treatment was equivalent to that observed in others who were greatly improved.

While it is recognized that symptoms in asthmatic individuals may be subject to considerable variation from day to day depending on a host of specific and nonspecific influences, and may frequently disappear spontaneously for varying periods of time, a number of observations in this series of patients indicate that oral cortisone exerted a profound influence on symptoms. The

rather prompt improvement which followed the initial dose of cortisone, relapse in certain instances when the dose was reduced to lower levels, the prompt recurrence of symptoms within a relatively short time after withdrawal, and the failure of placebo therapy to influence symptoms point to hormone therapy as the deciding factor in temporarily alleviating symptoms in these cases of asthma.

Effect on Atopic Dermatitis.—Five young adults (Table V) with severe atopic dermatitis received oral cortisone. Eczema had been present in these patients for periods ranging from 9 months to 18 years. Each patient gave a history of respiratory allergy which at the time of cortisone therapy was relatively quiescent. The eruption in each instance was in a particularly severe phase, and little improvement was occurring from local therapy at the time. The initial daily dose of cortisone was from 150 to 200 mg., and was administered in divided doses at 4- to 8-hour intervals for several days, and then gradually reduced and discontinued. The periods of therapy ranged from 6 to 16 days, with total doses of 600 to 1,550 mg. of the hormone being given. A reduction in eosinophiles ranging from 32 to 84 per cent occurred. A very dramatic clearing of the dermatitis was observed in 2 patients during the course of therapy. Another patient appeared to be partially improved, while the eczema in 2 others was not visibly altered during cortisone administration.

The favorable response which occurred in one patient was as follows:

TABLE V. EFFECT OF ORAL CORTISONE IN ATOPIC DERMATITIS

CASE NUMBER	AGE (YEARS)	SEX	CORTISONE		NUMBER OF DAYS OF THERAPY	THERAPEUTIC EFFECT			RECURRENCE (DAYS AFTER THERAPY)	
			TOTAL DOSE (MG.)	MAXIMUM DAILY DOSE (MG.)		DEGREE	INITIALLY NOTED (HOURS)	MAXIMUM OBTAINED (DAYS)		DEGREE
18	22	F	1550	200	16	poor	–	–	–	–
19	19	M	1300	200	10	poor	–	–	–	–
20	26	M	600	200	6	partial	72	4	–	–
21	22	F	700	200	6	complete	24	4	1	severe
22	16	F	700	150	7	complete	6	4	1	severe
			1200	150	15	complete	4	4	7	moderate

CASE 22.—A 16-year-old girl, a student, had developed an eczematous eruption involving the face and forehead 9 months previously. Except for brief periods of remission during 2 hospital admissions, and for a short time while away from home, the dermatitis persisted and responded poorly to local therapy. In the past, ragweed hay fever had been successfully treated by desensitization. In addition she was found strongly sensitive to house dust and molds. Oral cortisone was begun on an ambulatory basis with a total dosage of 150 mg. daily. Six hours after the first dose of 25 mg., improvement in the dermatitis occurred, progressing to complete disappearance of the eruption by the fourth day. Cortisone dosage was gradually reduced and discontinued on the seventh day with recurrence of the dermatitis 24 hours after the last dose of 25 mg. Within 48 hours it was as severe as it had been prior to therapy. Placebo tablets similar in appearance to oral cortisone were administered during the next 2 days without change. Oral cortisone was substituted on the third day in doses of 25 mg. every 4 hours with beginning improvement approximately 8 hours later. The dermatitis had entirely disappeared at the end of 4 days of therapy. During the next week the dose was reduced to 50 mg. daily and maintained at this level for an additional 2 weeks without recurrence of

eczema. In view of the previous remissions which occurred while away from her home surroundings, she left the city. Approximately one week after stopping the hormone, while away, the dermatitis recurred, although not in its original severity.

A second patient whose eczema cleared completely during a 6-day period of oral cortisone therapy experienced a relapse one day after treatment was stopped, and symptoms progressed to their original severity within a few days. Another patient appeared partially improved during oral hormone therapy, and has remained approximately the same for the period of 2 months which has elapsed since treatment. Since the changes observed in the latter case were not as dramatic or complete as those observed in the 2 patients described previously, it was difficult to be certain that cortisone was responsible for the partial remission.

One patient who failed to improve while on oral cortisone later received ACTH therapy in the hospital, and was moderately improved after this course of treatment, but relapsed almost immediately on leaving the hospital. Another patient who did not improve during oral therapy was subsequently hospitalized, at which time cortisone was given by injection. After a few days of such therapy the patient developed a severe psychic disturbance which required the discontinuation of hormone therapy.

Effect on Contact Dermatitis.—Two patients with eczematous contact type dermatitis involving exposed areas were given oral cortisone. The cause of the eruption in each of these cases had not been determined at the time cortisone was administered. Both were in an acute phase and it was felt that they were still in continued contact with the offending allergen. Bland local measures consisting of compresses of Burow's solution and a shake lotion which had been in use over a period of days with only limited improvement were continued during the period of cortisone treatment.

CASE 23.—A 38-year-old housewife developed an acute weeping eczematous eruption involving the face, eyelids, ears, neck, hands, and forearms 10 days before. A daily dose of 200 mg. of cortisone was given in amounts of 50 mg. at 6-hour intervals. Marked improvement occurred during the first 24 hours, and she was completely free of the eruption at the end of 48 hours. The dose was reduced in the usual manner and discontinued after 8 days, when a total of 1,100 mg. had been administered. Recurrence of the dermatitis about the eyelids was noted one day later, becoming more extensive during the next 3 days, although never reaching its original severity. She improved slowly on conventional therapy, and later investigation indicated that hair lacquer was responsible for the dermatitis in her case.

CASE 24.—A 48-year-old housewife had experienced recurrent episodes of dermatitis of the face and eyelids during the preceding 4 months. Cortisone in the amount of 200 mg. daily was started during an acute phase, with improvement noted during the next 48 hours. The dermatitis continued to improve during the next 5 days but did not entirely disappear. The dose was progressively reduced and discontinued after a total of 750 mg. had been administered. A severe flare-up of the dermatitis occurred 12 days later, but failed to be favorably influenced during a second course of oral cortisone similarly administered.

In the first case cited, it appeared that oral cortisone was responsible for the remission. In the second case the part that cortisone played in the initial improvement noted was doubtful.

Effect on Urticaria and Angioneurotic Edema.—Oral cortisone was administered to 4 patients with severe urticaria and angioneurotic edema. The

precipitating factor in 2 patients was considered to be sensitivity to penicillin. Both of these improved greatly during cortisone therapy. One remained well afterward, while the other relapsed shortly after the hormone was withdrawn. In 2 other patients with chronic urticaria and angioneurotic edema in whom the specific precipitating factors were not definitely determined, cortisone appeared to alleviate symptoms in one case only during the period when a high dose was being given, while in the other case symptoms were completely relieved during therapy, only to recur shortly after the hormone was discontinued.

CASE 25.—A 30-year-old housewife received penicillin for cellulitis of the elbow and one week later developed severe generalized urticaria and angioneurotic edema. Seven days after the development of symptoms, she was given 50 mg. doses of cortisone at 8-hour intervals. Marked improvement occurred after 24 hours, and the dose of cortisone was tapered off during the next 5 days, and discontinued after a total of 600 mg. had been administered. There was no recurrence of symptoms.

CASE 26.—A 38-year-old clerk developed urticaria, angioedema, and a bullous eruption of the legs shortly after a course of penicillin was given for a respiratory infection. After 3 months of continuous difficulty, a daily dose of 100 mg. of oral cortisone was given for 2 days without change in his condition. The dose was increased to 200 mg. daily on the third day, and marked improvement took place during the next 48 hours, with disappearance of lesions and healing of the bullous dermatitis. A total of 1,375 mg. was given during a period of 16 days. The patient experienced a recurrence of urticarial and bullous lesions 4 days after the hormone was stopped. A second course of cortisone by mouth, starting with 200 mg. daily, appeared to influence his condition favorably within the first 24 hours. The dose was progressively reduced until at the present writing he is taking 25 mg. of cortisone every third day without recurrence of his difficulty.

CASE 27.—A 23-year-old man, a factory worker, presented recurrent urticaria and angioneurotic edema of 6 months' duration which appeared to be precipitated by exposure to paint fumes encountered in his employment. During a particularly severe episode he was given placebo tablets for 3 days without improvement in his condition. This was followed by 200 mg. of cortisone daily with marked improvement during the next 24 hours. After 2 days at this dosage level, cortisone was reduced to 100 mg. daily, with recurrence of symptoms during the following 24-hour period. When the dose was again increased to 200 mg. he became symptom-free but again relapsed when the dose was reduced to 100 mg.

CASE 28.—A 68-year-old hypertensive woman with daily urticaria of 3 years duration, the cause of which was undetermined, was completely relieved after receiving 100 mg. of cortisone daily for 4 days. She remained completely free of urticaria during the next 11 days while the dose was progressively reduced and finally discontinued. A rather severe recurrence of urticaria developed 8 days after the last dose of cortisone.

SIDE EFFECTS

It is recognized that the clinical use of ACTH and cortisone in man is attended by the development of a number of hormonal and physiologic alterations apart from the therapeutic effect on the disease states for which they are being given. Effects on the metabolism of carbohydrate, fat, protein, water, in electrolytes may occur, as well as changes in the structure and function of the adrenal cortices. Untoward reactions during therapy with oral cortisone were apparently limited in this study by the relatively brief periods of administration at high dosage levels. The most common undesirable effect observed was fluid retention of a sufficient degree to produce edema. A rapid

gain in weight with development of edema of the face and ankles was detected in 3 women during the first 3 to 4 days of therapy at initial dosage levels, despite a diet low in sodium. Excretion of retained fluid occurred promptly in each case upon reducing the dose of cortisone during the next few days. Hormonal effects noted in this series were the occurrence of mild acne in one young woman and the delay in the menstrual periods of 3 others during the period of therapy. Acne disappeared and normal menstrual function was restored in a short time after cortisone was stopped in each instance. Psychic effects such as an exaggerated feeling of well-being or mild euphoria were frequently observed. In one patient, as already mentioned, a frank psychotic state developed during treatment with cortisone suspension shortly after a course of oral cortisone had been given. Mild gastrointestinal symptoms consisting of diarrhea in one case and epigastric distress in 2 others were reported during treatment.

DISCUSSION

It is evident from these observations in allergic disease that the effects of orally administered cortisone are very similar to those following the use of cortisone suspension administered intramuscularly,[1, 3] which have been previously described. The rather prompt alleviation of symptoms in many of these patients shortly after the oral hormone was begun and the rapid recurrences when the hormone was reduced in amount or withdrawn indicate that these changes were not merely spontaneous or coincidental, but were due to the influence of the adrenal cortical steroid. It has been our experience with the use of injectable cortisone suspension in severe hypersensitive states that 2 or 3 days of therapy are usually required before the full effect of the hormone becomes manifest. A somewhat more rapid clinical effect may usually be expected from the oral material. In many instances, symptomatic relief was attained within 6 hours of the initial dose, and a complete remission was present at the end of 24 to 48 hours of therapy. The effects of orally administered cortisone appear to be of shorter duration, and in the initial stages of therapy at least, it appears desirable to administer the hormone at intervals of 4 to 8 hours. These factors point to a rapid and rather complete absorption of cortisone from the gastrointestinal tract. There are undoubtedly some instances where a defect in absorption may delay or prevent the action of the material. In at least one instance in the present study, failure of oral cortisone to influence symptoms could be attributed to poor absorption from the gastrointestinal tract. In most instances where the oral material failed to induce a remission, such an occurrence could not be held accountable, since the eosinophile depression was of the same order at that which occurred in patients who were clinically improved during therapy. It is of course possible that higher doses of cortisone administered over longer periods of time may perhaps produce a more favorable response in some of these cases. There are, however, many instances where parenterally administered ACTH and cortisone fail to influence these conditions. In order to be able to explain adequately

the reasons for the failure of these hormones to affect symptoms in some instances, it would be necessary first to understand the mechanisms by which they exert their favorable effect on manifestations of hypersensitivity. While the influence of cortisone and ACTH on immune responses has been extensively studied, there is as yet no conclusive evidence pointing to the manner in which they affect these reactions. There is a considerable difference in the results of various investigators concerning the influence of these hormones on antibody formation. While earlier studies pointed to the enhancement of antibody production under the influence of increased adrenal cortical function,[5] later work has either failed to show any alteration in antibody or has shown an impairment in antibody formation.[6, 7] Experimental evidence indicates that adrenal cortical steroids or their increased production under the influence of ACTH does not interfere with antigen-antibody union.[8, 9] It is possible that there is an alteration in the abnormal tissue reaction that follows antigen-antibody union, although the mechanism of such an action has not been shown. While an increased level of adrenal steroids may increase the breakdown of histamine and reduce its rate of formation,[10] they do not prevent the local or general reactions of histamine as such.

Observations with both the oral and parenteral administration of cortisone indicate that the dosage requirements of the 2 forms are approximately equal. The more rapid onset of effect from the orally administered material and the relatively shorter duration of action suggest its initial administration in divided doses at frequent intervals. Improvement is maintained in many cases, even when the intervals are increased and the dosage reduced, but in some instances symptoms tend to recur at lower dosage levels. Since it is apparent that the remissions which are induced by a course of oral cortisone therapy are relatively brief, efforts to prolong the improvement by continuing to administer small amounts over a longer period of time may seem worthy of trial. Our experiences to date with extended dosage schedules are too limited to warrant any conclusions at the present time. In using cortisone over a long period, however, it should be realized that adrenal function may be depressed for some interval after the hormone is stopped. The more frequent occurrence of metabolic alterations and hormonal side effects must also be given consideration when longer periods of therapy are contemplated.

While there is little doubt that oral cortisone, as well as ACTH and parenteral cortisone, frequently produce dramatic improvement in severe hypersensitive states, their place in the management of chronic allergic disease is yet to be clarified. It is possible that the immediate advantage gained from their use may at times be outweighed by other disadvantages when the long-term character of the disease is considered. We have gained the impression, since using these agents in chronic asthma, that symptoms may become more severe and difficult to manage after successive courses of either hormone have been given. In view of the limited knowledge concerning the manner in which ACTH and cortisone produce their effect in allergic disease, it would seem ad-

visable, for the present at least, to limit their use to periods of extreme distress, where symptoms are not readily controlled by other methods at our disposal.

SUMMARY

1. Orally administered cortisone is readily absorbed from the gastrointestinal tract, and in comparable dosage affects allergic diseases in much the same manner as the saline suspension of cortisone given by intramuscular injection.

2. A moderate depression in blood eosinophiles is apparent 4 hours after a single 25 to 50 mg. dose of oral cortisone. Maximum depressions are usually attained during the second to fourth day of therapy.

3. Symptoms of hay fever, asthma, urticaria, atopic dermatitis and contact dermatitis are often completely relieved during oral cortisone therapy. The onset of such improvement appears to be more rapid than with cortisone suspension administered by injection, but the therapeutic effect of each dose of the oral material is dissipated more rapidly.

4. The effects of oral cortisone, although striking in many cases, are of relatively brief duration, and chronic symptoms recur shortly after withdrawal of the hormone. Close supervision of the patient is necessary to avoid the known side effects of adrenal hormone therapy.

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ACTH IN RAGWEED POLLINOSIS*†‡

A Histologic, Immunologic, and Clinical Study

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RECOGNITION of the fact that the clinical manifestations of allergy can be suppressed by ACTH and adrenal corticoids made it desirable to investigate their possible mode of action. Seasonal allergic conditions—well defined with regard to onset, causative agents and shock tissue—lend themselves well to this particular problem; in fact, they have been investigated in various clinical studies.

The limitations of such studies are obvious. Even without the use of ACTH the interpretation of tissue changes in allergic individuals is complicated by the variability of the factors involved, for example, the differences in the sensitivity of the various patients or the unpredictable changes of exposure to atmospheric allergens. In addition, the relationship between eosinophils in circulating blood and tissues has remained a controversial issue. The advent of ACTH has made it more so. The multiple action of these hormones may interfere in several direct and indirect ways with the distribution of eosinophils. For this reason, every attempt to interpret the effect of ACTH on allergic tissue is a pilot project subject to re-evaluation.

The major difficulty in attempting to evaluate the effect of ACTH on the tissues of the allergic patient is due to the fact that we have none but very gross indicators for the action of the hormones involved on a patient in general and specifically on connective tissue. Conventional methods of fixation and staining give little information about the enzymatic changes which may possibly account for the action of pituitary adrenal hormones in allergic disease. The clinical effect of the hormones, on the other hand, has been reported by several investigators. It seemed sufficient therefore to deal but briefly with the effect of ACTH on symptoms.

METHODS

Selection of Patients.—Thirty-five patients from the Allergy Clinic of the University of Illinois College of Medicine were chosen for this study. Of these, 34 cooperated throughout the experiment. While the symptoms in all cases were due primarily to ragweed pollen, 13 had minor complaints attributable to mold sensitivity. The age distribution was from 22 to 74 years with only 6 above 60 years of age. Fourteen were males and 20, females. Eight of the 34 were selected at random to receive placebo injections. Seven of these had asthma complicating their hay fever. The remaining 26, consisting of 20 with asthma and 6 with nasal allergy only, received ACTH injections.

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‡A generous supply of Corticotropin was donated for this study by Wilson Laboratories, Chicago, Ill.

Medication.—Twenty-six patients received Corticotropin (Wilson), 20 mg. dissolved in 1 c.c. of phenol water of an approximate pH of 3, intramuscularly at 8:30 A.M., 12:30 and 4:30 P.M. through 4 consecutive days (August 28-31).* Eight controls were given injections of the solvent without Corticotropin at the same intervals. No other medication was allowed during the period of observation.

Clinical Evaluation.—Two physicians (H. L. and N. J. E.) were independently responsible for interviewing the patients and evaluating the results of therapy. In this, as in all the other phases of the experiment, a code was used so that the examiners did not know which were "control" and which were treated patients. All were questioned and examined on August 24 for the degree of symptoms. They were next seen on August 27, and daily thereafter, until the end of the experiment. The severity of symptoms reported by the patient and the findings of the physicians were recorded. While it was recognized that only a rough approximation of clinical effects could be determined in such a short-term experiment, it is of interest that the graphs of symptoms made for each patient by both physicians were quite similar in almost all cases.

Determination of Circulating Eosinophils and Urinary 17-Ketosteroids.—In order to ascertain the activity of ACTH in our patients, 2 laboratory tests were carried out: (1) The number of circulating eosinophils before, during, and after the administration of Corticotropin and placebo was determined. For this, Randolph's[1] method of absolute chamber counts using aqueous propylene glycol diluting fluid was used throughout the study and proved satisfactory. Heparinized blood obtained by venopuncture was used for the counts. These were made before the beginning of the experiment, at the time of the first biopsy, before ACTH, 4 hours after ACTH, 16 hours after the last dose of ACTH, and at the time of the second biopsy. (2) The urinary excretion of 17-ketosteroids before treatment and throughout the period of observation was determined by Cahen and Salter's[2] method for the estimation of 17-ketosteroids in aliquots of 24 hour urine specimens.

Skin Tests.—A freshly prepared extract of giant and short ragweed pollens which contained 50 per cent glycerol for preservation was diluted with normal saline solution for intradermal tests. The first dilution for skin tests contained 0.001 mg. total nitrogen per milliliter. This was diluted at tenfold intervals so that the final or sixth dilution was at a concentration of 0.00000001 mg. total n. per milliliter. The 6 dilutions and buffered normal saline solution which served as control were injected intradermally in 2 rows on the volar surface of the forearm. Special care was exercised in attempting to raise uniform wheals about 2 mm. in diameter. Ten minutes after the injection the wheals were examined, outlined with India ink, and transferred to Scotch tape for permanent recording. Tests were made on August 27, 24 hours before starting treatment, and again with freshly prepared solutions on September 1, 16 hours after the last injection had been given. The wheal sizes and the end points of the reactions recorded on the 2 days were compared in each patient.

*It is difficult to establish the potency of Corticotropin since the available biologic tests show considerable variation and, furthermore, refer only to one of several specific functions. Dr. David Klein, Director of Research of Wilson Laboratories, estimates that Corticotropin, unit per unit, is at least as strong, probably twice as strong as Armour's LA-1-A.

Reagin Titration.—Serum for reagin titration was obtained before treatment (August 27) and again 16 hours after the last injection (September 1). The sera were tested for sterility, labeled in code by a technical assistant, and stored in the refrigerator. One of the sera from an untreated patient was lost during preparation. The 7 remaining pairs of "control" sera and 8 pairs of sera from the ACTH-treated patients were set aside for titration.

The first dilution for titration consisted of 1 part of serum plus 3 volumes of sterile saline solution. From this, dilutions were made at twofold intervals to a total of 9 (1:1024). Hospitalized patients with no history of allergy whose skin did not react to ragweed and who were proved receptors were selected for passive transfer. Two pairs of sera were injected in 4 corresponding rows of 9 dilutions on the back. Forty-eight hours later these were tested by the intradermal injection of approximately 1/100 ml. of ragweed solution containing 0.01 mg. total nitrogen per milliliter. The sizes of the wheals produced by the corresponding dilutions of the paired sera were compared at the end of 20 minutes.

Histologic Studies.—On August 24, 4 days before any injections were started, a tissue biopsy about 4 mm. square was taken with a Myles anterior biting forceps from the anterior overhang of the left lower turbinate. A 2 per cent pontocaine solution was sprayed into the nose 5 minutes before this procedure. The pain and bleeding were so slight that full cooperation was given by all patients for the second biopsy. This was taken from the opposite inferior turbinate on September 1, the morning following the fourth day of treatment, 16 hours after the last placebo or ACTH injection.

The tissues removed were fixed by the Altmann-Gersh freezing-drying method.[3] This technique requires the rapid freezing of the tissue immediately after removal in iso-pentane chilled by liquid air to approximately –150° C. Without allowing any thawing, the tissue is dehydrated in vacuo at about –30° C. By this method certain morphologic and chemical artefacts are avoided. Protein denaturation is largely prevented and shifts in organic and inorganic tissue components are minimized. Enzyme activity is arrested. The use of this technique permitted the study now in progress of the histochemical changes.

After the tissues were completely dehydrated at –30° C., they were prepared for sectioning by embedding in paraffin according to the usual technique. Sections cut at 6 μ were stained by hematoxylin and eosin for routine histologic studies and by Giemsa for the study of eosinophils. The sections were examined and described independently by 2 of us (B. Z. R. and E. A. McG.).

In addition to the routine examination, each tissue was studied in detail for the following: (a) evidence and degree of edema, (b) the number, location, and types of round cells and neutrophils indicative of "inflammation," (c) eosinophils, their location, number, and condition. The importance of eosinophils in allergy led to critical evaluation of each tissue regarding the number of intact and destroyed cells. The average number of intact eosinophils and of irregular granular clumps for 10 high-power fields were determined (4 mm. objective ×47.5, eyepiece ×10). Cells that had a nucleus, even though pyknotic or fragmented, and granule masses of round or oval shape suggesting a cell were

counted as "intact eosinophils." Irregular or angular masses of granules of approximately the area of an eosinophil were counted separately. Granules present diffusely were noted but not counted as units. The finding of ruptured eosinophils led to a critical search for evidence of phagocytosis.

RESULTS

Symptomatology.—Despite the difficulty offered by the unusual pollen counts* it was apparent to both physicians (H. L. and N. J. E.) who were responsible for the clinical study that improvement occurred in 9 of the 26 patients who received ACTH injections. The 8 "controls" showed no improvement either subjectively or objectively. Seven of the 26 patients who received ACTH and 2 of the 8 "controls" had mild symptoms of sleeplessness, palpitation, insomnia, nervousness, hyperactivity, muscular cramps, and dizziness. These symptoms were transitory and were not significantly different in the 2 groups.

Systemic Effects of ACTH.—The initial levels of circulating eosinophils in both treated and control groups showed wide variations. With few exceptions, the response to the injection of ACTH ("Thorn Test") was satisfactory. In a significant number of patients circulating eosinophils were still depressed 16 hours after the last injection of ACTH† The eosinophil counts have been analyzed in accordance with the criteria set forth in a recent paper by Best and Samter.[4]

In our patients, the increase in the amount of excreted 17-ketosteroids corresponded roughly to the intensity of the eosinophilic response. Excretion of 17-ketosteroids, however, is an indirect rather than direct indicator of ACTH activity, influenced by age and sex, a fact which limits its significance. With the method which we have used, normal values for men are from 12 to 15 mg., for women from 8 to 11 mg. 17-ketosteroids in 24 hours.

Skin Tests.—A comparison of the intradermal skin tests made on August 27 and September 1 indicated fairly close agreement in all patients not only in the size of the wheals produced by the various ragweed dilutions but also in the end points of the reaction on the 2 days. The patients were graded on the basis of the last positive reaction obtained and classified in groups of ascending sensitivity from 1 to 6. On this basis, Group 1 (the least sensitive) was composed of one patient, Group 2 of three, Group 3 of eight, Group 4 of six, Group 5 of thirteen, and Group 6 (the most sensitive) of three. The data obtained were used in an attempt to determine the existence of certain correlations which will be discussed.

Reagin Titration.—The reagin titrations were done according to the dilution method previously described. The number of the highest serum dilution

*The ragweed pollen count (O. C. Durham) for the 24 hours preceding August 28, the morning when the injections were started, was 180 gr. per cu. yd. per 24 hours. The pollen counts thereafter were 53 (8-29), 118 (8-30), 16 (8-31), and 48 (9-1). It is evident that the daily ragweed pollen counts in the Chicago area in 1950 were not only low, but also an unusual pattern for this period.

†On theoretical grounds, one might reason that in view of this prolonged effect a longer interval between last ACTH injection and tissue biopsy should have been selected to avoid surgical complications. In fact, however, we observed neither undue bleeding, delayed healing, nor scar formation.

to which a patient gave a positive reaction was used to classify him for comparative reagin concentration. Here, as in the direct skin tests, the paired sera of the 7 controls and 8 treated patients had essentially the same titer before and after injections. The one difference of any significance occurred in a patient who had been given placebo injections. The September 1 serum had a definitely higher titer than that of August 28. In view of these results it appeared unnecessary to make further comparison of pre- and posttreatment sera. Therefore, the sera drawn only on August 27 were used for titration by the dilution method for the purpose of classifying all patients on the basis of reagin concentration. The results of this study will be discussed under "correlations."

Histopathology.—The histopathologic changes will be reported in detail at a later date. A brief summary of the findings is given here.

Most of the sections showed definite evidence of edema not only of the connective tissue but of the cells as well. In general there was a tendency for the degree of tissue edema to be less in the second than in the first biopsy, although the incidence of this finding was no higher in the group treated with ACTH than in the controls. Hyperemia and dilatation of lymphatics was essentially unchanged in the majority of cases, while the appearance of fibrosis was more marked in many of the second biopsies.

Cellular infiltration was almost limited to the portion of the submucosa between the basement membrane and the glandular layer. This finding coincides with that described by Hansel.[5] The degree of cellular infiltration varied from slight to marked. In this respect, as in the edema, there was no uniformity to differentiate the "control" cases from those treated with ACTH.

The cells making up the infiltrate consisted of lymphocytes, plasma cells, and eosinophils. The plasma cells were unusually large. The mononuclear inflammatory cells appeared to have diminished in number in many of the tissues removed on September 1 and to have increased in only a few. The eosinophils varied in number from only an occasional cell to as many as 150 in a high-power field. Here, also, there was no characteristic pattern of difference between treated and control patients. While most of the patients had approximately the same number of eosinophils in the tissue in the second specimen as were present in the first (Tables I and II), almost as many displayed fewer eosinophils. These corresponded roughly with those whose edema and infiltration of mononuclear cells seemed to have diminished. In many of the tissues there was a high proportion of disrupted eosinophil cells. These were characterized by diffuse granules in the tissue, by clumps of granules, and by evidence of nuclear degeneration in otherwise intact eosinophil cells. Generally, the disruption of eosinophils is not noted in tissues prepared by the freezing-drying technique.*

In many of the tissues, spindle-shaped aggregates of eosinophilic granules were present, associated with fibroblast nuclei, and giving the impression of fibroblasts containing eosinophilic granules. These were found along blood vessels as well as diffusely among the collagen fibers. Their arrangement along

*Personal communication from Dr. I. Gersh: "Extensive experience with the Altmann-Gersh technique indicates that it does not cause disruption of eosinophils with the resulting diffusion of cytoplasmic granules."

the borders of blood vessels paralleling the endothelial cells suggested a structural relationship to the vessels. Their appearance in relationship to the vessel walls resembled closely the "pericytes" described by Maximow.[6] While these fibroblast-like cells were present in sections containing a high eosinophil concentration, they were also numerous and easily found in tissues containing few intact eosinophils but many eosinophilic granules. In addition, a few typical macrophages containing eosinophilic granules of varying sizes in their cytoplasm could be identified.

TABLE I. COMPARISON OF EOSINOPHILS IN THE BLOOD AND NASAL TISSUE OF RAGWEED-SENSITIVE PATIENTS (UNTREATED CONTROLS) OBTAINED AT A FOUR-DAY INTERVAL DURING THE RAGWEED SEASON

PATIENT NUMBER	DATE	PERIPHERAL TOTAL EOSINOPHIL COUNT	TISSUE EOSINOPHILS	
			RANGE/HPF	INTACT EOSINOPHILS AVERAGE OF 10 HPF
1	August 24	27	0	0
	September 1	50	0	0
2	August 24	1010	150+	150+
	September 1	1120	*	*
3	August 24	55	0-6	1.2
	September 1	144	0-2	0.6
4	August 24	226	5-8	6.0
	September 1	167	10-23	14.2
5	August 24	299	1-6	2.4
	September 1	333	0	0
6	August 24	155	0	0
	September 1	72	1-2	0.6
7	August 24	211	0-12	3.8
	September 1	100	0-4	2.8
8	August 24	488	2-16	8.5
	September 1	511	0-3	1.0

*Unsatisfactory fixation of tissue.

CORRELATIONS

A variety of factors related to the allergic state affect the conditions in the shock tissue. They may be considered under 3 general headings: (a) those factors whose effect on the shock tissue is generally known and well established, (b) certain suggested relationships established through this study, (c) factors to be considered whose effect is unknown.

The effect of the inhaled allergen on the nasal mucosa has been long established. Inhalation of pollen produces edema and cellular infiltration of the submucosa with eosinophils as the most characteristic cell. The decrease in the edema generally observed in the tissues of September 1, when compared with that in the sections of August 24, is at least in part due to the lower concentration of pollen on September 1 (48 gr. per c. yd. per 24 hours) compared to the much higher count on August 24 (247 gr. per c. yd. per 24 hours). This is suggested by the finding that in 3 of the 6 pairs of control tissues available for study the edema was less in the material removed on September 1 as compared with the material removed on August 24. Thirteen of the 26 patients treated with ACTH likewise showed diminished edema in the tissues of September 1. While the ACTH administered to these patients may have been

TABLE II. COMPARISON OF EOSINOPHILS IN BLOOD AND NASAL TISSUE OF RAGWEED-SENSITIVE PATIENTS OBTAINED AT A FOUR-DAY INTERVAL DURING THE RAGWEED SEASON BEFORE AND AFTER ACTH INJECTIONS

PATIENT NUMBER	DATE	PERIPHERAL TOTAL EOSINOPHIL COUNT	TISSUE EOSINOPHILS	
			RANGE/HPF	INTACT EOSINOPHILS AVERAGE OF 10 HPF
9	August 24	388	28-95	65.4
	September 1	189	0-4	1.5
10	August 24	832	0-3	0.7
	September 1	278	0-2	0.5
11	August 24	410	3-6	4.6
	September 1	133	0-4	1.2
12	August 24	88	0-2	0.3
	September 1	78	0-1	0.2
13	August 24	311	0-2	0.2
	September 1	111	0	0
14	August 24	266	0-7	1.5
	September 1	17	0-2	0.7
15	August 24	222	0-17	5.6
	September 1	200	4-21	12.3
16	August 24	155	0 8	3.4
	September 1	78	0-4	1.1
17	August 24	39	0-5	1.9
	September 1	78	0-15	4.0
18	August 24	311	0-1	0.4
	September 1	244	0	0
19	August 24	277	0-1	0.1
	September 1	44	0	0
20	August 24	167	0-2	0.2
	September 1	11	0	0
21	August 24	410	1-5	1.7
	September 1	28	0-3	1.4
22	August 24	255	1-66	10.8
	September 1	28	4-45	25.0
23	August 24	810	2-233	10.3
	September 1	122	16-29	25.5
24	August 24	666	2-9	3.9
	September 1	222	1-11	3.7
25	August 24	611	0-6	2.3
	September 1	78	4-7	5.6
26	August 24	66	0-2	0.6
	September 1	56	0-1	0.2
27	August 24	133	7-29	22.0
	September 1	78	0-3	2.0
28	August 24	532	17-32	23.4
	September 1	66	0-3	0.7
29	August 24	410	0-1	0.2
	September 1	45	0-1	0.2
30	August 24	543	10-35	15.2
	September 1	0	0-1	0.4
31	August 24	377	10-27	22.0
	September 1	100	3-10	3.8
32	August 24	281	0-17	5.8
	September 1	39	10-19	14.1
33	August 24	201	0-1	0.2
	September 1	89	0-4	1.4
34	August 24	244	0-6	1.8
	September 1	100	4-11	7.8

effective in this change, it is not unlikely that the change in pollen concentration also may have been an important cause for the decrease in tissue edema.

Tissue eosinophilia is affected not only by the inhaled antigen but by other factors, some identifiable, others unknown. While inhalation of pollen

is known to cause an increase in the number of tissue eosinophils, the rise is not proportional to, or even consistent in this series with, the pollen concentration. Although the pollen count on August 24 was high, the concentration of eosinophils in the 34 original biopsies varied widely. Nor was the decline in pollen count of September 1 associated with a consistent change in tissue eosinophilia in either the control or treated patients. It is our impression that the change in eosinophil content of the tissue may be due in part to the rate of their destruction and removal from the tissue. The cause of such destruction, in vivo, is at present obscure.

The concentration of eosinophils in the nasal tissues may be related to the peripheral eosinophil count (Tables I and II). All patients were classified in ascending groups according to their peripheral eosinophil count arranged at intervals of 100 cells. The average peripheral cell count was determined for each group. The average tissue eosinophilia per high-power field for the patients in each of these groups was likewise determined. The rank correlation coefficient[7] between the average peripheral eosinophil count in the groups mentioned versus the average tissue eosinophil count per high-power field was 0.47 ± 0.18. This correlation is significant ($P < 0.05$) within the limitations of data in such a small group of patients.

Similarly, the degree of skin sensitivity to ragweed pollen seemed to be related to the concentration of tissue eosinophils. As noted previously, all patients were graded and classified in groups corresponding to the positive skin reaction with the highest pollen dilution. On this basis the patients were classified into 6 groups according to skin reactivity. When a comparison was made between the number of patients in each of these groups and the average tissue eosinophil count per high-power field for each corresponding group, a statistically significant correlation was again established. This coincidentally was the same as that determined for the average peripheral versus the average tissue eosinophilia, 0.47 ± 0.18 ($P < 0.05$). In addition, the findings of the degree of skin sensitivity versus the average peripheral eosinophilia suggested a relationship which was just barely significant (rank correlation coefficient 0.35 ± 0.17) ($P = 0.05$).

The many factors involved in these biologic variants as well as the small samples studied do not permit more than tentative conclusions. Within these limitations one may say that in the presence of an adequate amount of inhaled antigen the higher the skin sensitivity, the greater the peripheral eosinophil count, the greater the tissue eosinophilia.

Surprisingly, the reagin titration was not proportional to the skin sensitivity. This differs from the findings of Levine and Coca,[8] who determined that the reagin titer was generally proportional to the skin sensitivity in 35 pollen-sensitive cases that they studied.

DISCUSSION

The clinical findings were inconclusive. In retrospect it appears that the general euphoria, attributable not only to the effect of ACTH but to the holiday spirit of group cooperation, led to lessened expression of complaint.

When this study was planned, the effect of ACTH on skin reactivity had been studied in a few cases.[9, 10, 11] The failure of ACTH to change skin reactivity and serum reagin concentration in the 26 cases reported here confirms the findings of others that ACTH does not affect the antigen-antibody reaction.

Histologic study of the nasal tissues fails to show any changes that might be attributable to the effect of ACTH. This confirms Bordley's study[12] of polypoid tissue from 5 patients. While the biopsies of September 1 were removed 15 to 16 hours after the last dose of ACTH had been given, the low peripheral eosinophil count present that morning in most of these patients (Table II) indicated that the physiologic action of ACTH was effectively present. Additional studies with histochemical methods now in progress may show tinctorial changes of ground substance in treated patients. At present, however, we can add nothing to Hench's statement[13] that the effect of corticoids is on the pathologic physiology rather than on the pathologic anatomy or on the etiological factors.

The histologic changes shown, especially in the eosinophils, are of interest as they apply to the shock tissue of the immediate wheal type sensitivity. The disruption of eosinophils was found in all 3 groups of tissues, pretreatment, posttreatment and control cases, with no characteristic difference in the pattern. While further study using formalin-fixed tissues to compare with the freezing-drying technique may be necessary to determine whether some of the eosinophil damage may be due to artefact, the presence of eosinophilic granules in fibroblast-like cells and, indeed, within typical macrophages indicates that at least part of the destruction of eosinophils is an in vivo process.

The studies suggest the following possibilities. Tissue eosinophilia might depend on 3 factors: (a) the number and rate of fresh eosinophils delivered to, or originating in, the tissues (some evidence exists that peripheral eosinophilia and tissue eosinophilia are correlated), (b) the rate of their destruction, and (c) the rate of removal of the products of disintegration. It is interesting that hardly a tissue in this series failed to contain evidence of destroyed eosinophils.

SUMMARY

1. Nine of 26 ragweed-sensitive patients who received 60 mg. ACTH daily for 4 days showed clinical improvement.

2. None of the 8 patients who received placebo injections were improved.

3. Neither the reagin titer nor skin sensitivity to ragweed pollen were altered by treatment with ACTH for the indicated period.

4. Suggestive evidence was found that there is a correlation between the degree of skin sensitivity, the peripheral eosinophilia, and the tissue eosinophilia.

5. No change in histologic characteristics attributable to the use of ACTH could be demonstrated. Edema and eosinophils in various stages of disintegration were found in pretreatment, posttreatment, and control biopsies.

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THE EFFECT OF ADRENALECTOMY ON EXPERIMENTAL HYPERSENSITIVENESS*†

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THIS paper deals with the effect of adrenalectomy on experimental hypersensitiveness. It includes observations of anaphylaxis, precipitin formation, the Arthus phenomenon, and diffuse vascular disease (collagen disease) induced experimentally by sensitization with horse serum. There has been a great deal of interest recently in the role played by the adrenals in immunologic phenomena. The clinician has likewise been concerned with the therapeutic effect of adrenal cortical hormones in the treatment of certain allergic and collagen disorders. It is for these reasons that we have undertaken to find whether, and to what extent, the removal of the adrenals affects such phenomena and to speculate on the possible mechanisms whereby this takes place.

LITERATURE

The precipitin titer which develops following experimental sensitization usually is an index of the degree of anaphylactic sensitivity.[1] Similarly, the Arthus phenomenon develops more easily and more extensively following a challenging dose of antigen in more highly sensitized animals.[2] Diffuse vascular changes or collagen disease have been induced experimentally by Rich and Gregory[3] through sensitization. Moore and associates[4] also produced these lesions by sensitization with horse serum and egg white, and Criep and Mayer[5] with (Lederle's) streptococcus toxin. However, similar pathologic changes may be brought about by feeding lipids to dogs[6] or, as Selye demonstrated[7, 8] under laboratory conditions as a result of stress and shock. Under these latter circumstances, the development of the condition is closely related to the function of the adrenal glands.[7, 8]

A review of the literature indicates that there is no general agreement as to the effects of adrenalectomy on the antibody titer of sensitized animals. Thus Gates,[9] in 1918, reported that guinea pigs immunized with typhoid bacilli or hen corpuscles showed little change in antibody titers if the animals were partially adrenalectomized. Jaffe and Marine,[10] and Také and Marine[11] reported higher hemolysin and agglutinin titers in adrenalectomized rabbits and rats. Murphy and Sturm[12] a few years later found a rise in hemolysin titers in adrenalectomized rabbits, as well as hypertrophy of the lymphoid tissue when immunized with sheep cell antigens. However, in a more recent paper, Thatcher, Houghton, and Ziegler,[13] working with cats and rabbits and employing sheep cells as antigen, failed to corroborate these findings. They concluded that little change in precipitin titers occurred in their series of sensitized and adrenalectomized animals as compared with the controls. However, adrenalectomized animals appear to be more vulnerable to anaphylaxis than normal animals and also are more sensitive to histamine shock.[14, 15]

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There has also been much controversy over the effect of adrenocorticotropic hormone and adrenal cortical extract on antibody titers. White and Dougherty[16] indicated that the administration of these hormones in the intact animal causes a rise in antibody titer, presumably through lysis of lymphocytes, whereas no such change occurred in the adrenalectomized animal. Fischel, Le May, and Kabat,[18] using the quantitative technique in intact rabbits, could not confirm these findings. Eisen and associates,[19] working with adrenalectomized rats, found the same antibody titer after A.C.E. (adrenal cortical extract) administration as seen in adrenalectomized animals not receiving this hormone. They further stated that A.C.E. does not produce a prolonged effect on the formation or release of antibodies. Similar conclusions were reached by DeVries[20] who discovered that ACTH although producing lymphocytopenia, does not cause a release of antibodies into the circulation. Furthermore, no rise in gamma globulin has been found to occur after treatment with ACTH in man[21-25]; however, quantitative antibody titers were lowered during sensitization with pneumococcus vaccine while rabbits were receiving ACTH or cortisone. The titers also fell when cortisone or ACTH was started 14 days after sensitization.[58] Decreased mononuclear cells and atrophic changes in the lymph nodes were found. On the other hand, the administration of adrenal cortical extract in adrenalectomized animals, according to some, results in greater resistance to shock.[26] And yet, others[27] stated that anaphylaxis cannot be prevented in the intact guinea pig by pretreatment with ACTH. The administration of ACTH or cortisone to tuberculin-sensitive guinea pigs inhibits the local delayed reaction.[28] There seems to be doubt as to whether this takes place in man[29, 30]; however, recent work has indicated that a decrease in induration and necrosis of skin tests resulted while patients were receiving ACTH and cortisone. Skin tests with purified protein derivative (tuberculin) and beta hemolytic streptococci were used. Positive skin tests and rebound phenomena were noted after withdrawal of these hormones.[59] It has also been shown that ACTH inhibits the development of perivascular and cardiac lesions in rabbits sensitized with horse serum, as shown by Berthrong, Rich, and Griffith,[31] who gave ACTH to rabbits while sensitizing them.

It is established[16-20] that there is a fall in circulating lymphocytes in rabbits and a discharge of splenic lymphocytes in rats[32] after the administration of ACTH and A.C.E. Likewise, in man, this hormone leads to a fall in circulating eosinophiles and lymphocytes with a rise in neutrophiles.[24, 33] The lymphocytopenia which results from stimulation of the adrenal cortex is thought to be brought about by those cortical steroids which have an oxygen on carbon 11.[24, 34-36]

ACTH and cortisone have been used effectively in clinical allergy[57] and, with varying degrees of success, in some of the collagen diseases. In some of these conditions, notably in periarteritis nodosa, lupus erythematosus disseminatus, and in the rheumatic conditions, these hormones appear to tide the patient over the acute and fulminating phase of the disease.[37-41] In other collagen diseases, especially in scleroderma, the therapeutic effects seem negligible.[33]

The following study was undertaken with rabbits to determine the effect of adrenalectomy on the production of various phenomena of anaphylaxis, such as precipitin titers, the Arthus phenomenon, as well as the effect on the production of diffuse vascular disease. In addition, splenic weights and total white blood cell counts and eosinophil counts were compared in these adrenalectomized animals with control animals.

METHOD OF STUDY

A total of 57 rabbits was used in this study. These were divided into 7 groups for the purpose of using adequate controls. In this manner it was possible to compare the results obtained as a result of sensitization of adrenalectomized rabbits maintained on desoxycorticosterone (Doca) with the following groups of animals: (1) those which were adrenalectomized and sensitized but received no Doca; (2) those which were adrenalectomized, received Doca, but were not sensitized; (3) those which were adrenalectomized but received no Doca and were not sensitized; (4) those which were not adrenalectomized but received Doca and were sensitized with horse serum; (5) those which were sensitized, not adrenalectomized, and received no Doca; (6) and the last group of animals which were given Doca but were neither adrenalectomized nor sensitized.

Adrenalectomy was performed in 2 stages. The left adrenal was removed first via a lumbar-paraspinal approach. One week to 10 days later the right adrenal was removed in a similar manner. Ether anesthesia was used throughout and intravenous saline solution was given during both procedures. Immediately after operation all animals, controls included, were given 0.2 c.c. per kilogram of adrenal cortical extract and 0.1 mg. per kilogram of Doca each for one dose. Subsequently as has been indicated, some received Doca and others did not. In the Doca-treated groups, Doca was continued in doses of 0.5 mg. every other day. At the end of 7 to 10 days following the last stage operation, the animals were sensitized.

Sensitization was carried out according to the method of Rich and Gregory,[3] and modified as described by us elsewhere.[42] Ten milliliters of horse serum per kilogram were given intravenously 7 to 10 days following the last stage adrenalectomy. The second dose of 1 ml. was given intravenously on the seventeenth day, and the third dose of 10 ml. per kilogram of horse serum was administered on the nineteenth day.

The Arthus phenomenon was performed on the twelfth to fifteenth day after the initial injection of horse serum. It was produced by injecting 1 ml. of horse serum, partly intracutaneously and partly subcutaneously.

The animals were sacrificed by air emboli, if they did not die previously, on the twenty-sixth to the thirty-first day after initial sensitization. Blood for precipitin titer determination was drawn from the inferior vena cava. The antibody dilution method was used for the determination of the precipitin titer as described in a previous paper.[42] Briefly, this consists of placing 0.2 ml. of horse serum diluted 1-10 into Kahn tubes and mixing it with varying dilutions of rabbit serum in a volume of 1 ml. The mixtures were allowed to stand at room temperature for one hour and then were placed in a refrigerator for 48 hours.

The tube containing the highest dilution of rabbit serum showing a precipitate was taken as the final titer reading.

Microscopic sections of heart, lungs, spleen, pancreas, adrenal (where possible), and kidney were prepared with hematoxylin and eosin after previous fixation in formalin. Total white blood cell and differential counts before and after adrenalectomy were done in the usual way with 0.1 N hydrochloric acid and Wright's stain, respectively. The eosinophile counts were done on blood from the ear vein and prepared according to the method of Thorn and associates.[43] The total white blood cell counts, differential counts, and eosinophile counts were performed before and after adrenalectomy but before sensitization. The splenic weights were taken after the animals were killed or had died and were compared with the total body weights of the animals. These 2 figures were compared with normal control animals.

RESULTS

Group 1.—This group consisted of 32 rabbits. These animals were adrenalectomized and maintained on Doca. Sensitization by the technique just referred to was begun 7 to 10 days after the last stage adrenalectomy. Following are the results observed in this first group of animals.

Precipitin titers: A total of 20 rabbits in this group was studied for precipitin titers. Three animals showed titers of 1-100,000; one, 1-50,000; three, 1-20,000; three, 1-10,000; two, 1-5,000; one, 1-2,000; four, 1-1,000; one, 1-200; two, 1-50 (Table I). When these titers are compared with the controls in group

TABLE I. PRECIPITIN TITERS

TITERS*	NUMBER OF RABBITS	
	CONTROL	ADRENALECTOMIZED
1-100,000	0	3
1-50,000	0	1
1-20,000	0	3
1-10,000	0	3
1-5000	0	2
1-2000	2	1
1-1000	1	4
1-500	5	0
1-200	2	1
1-100	1	0
1-50	2	2
1-10	1	0

This table indicates a comparison of precipitin titers in the control or intact sensitized animals and the adrenalectomized sensitized animals.

*All sera tested were obtained at post-mortem.

VI (sensitized but not adrenalectomized) it appears clear that adrenalectomy causes a rise in the precipitin titer of sensitized rabbits. Four animals in Group I had lower titers than the rest (three, 1-50, and one, 1-1,000) but this can be explained since these were part of the group of 7 who developed fatal anaphylactic shock during the second sensitizing dose of horse serum. The sera tested were obtained at post mortem. It may be that most of the circulating antibodies were absorbed by the antigen and were thus not measurable by our technique. The precipitin titers of the other 3 rabbits however, were unaffected.

Arthus phenomenon: Of the 32 adrenalectomized animals in this group in which the Arthus phenomenon occurred, 16 showed markedly positive reactions. These were characterized by extreme redness, swelling, and necrosis of the local skin site, as well as by the rapidity and consistency of development. In a control group of 10 intact but sensitized rabbits the Arthus phenomenon occurred in 7, but to a marked degree in only 1 of the rabbits. Microscopic sections of the lesions in the adrenalectomized group showed marked necrosis of the cells of the uppermost epidermis. The underlying dermis was packed with a cellular exudate consisting of lymphocytes, polymorphonuclear leucocytes, eosinophiles, and plasma cells (Fig. 1). The collagen fibers showed fibrinoid degeneration and even the underlying muscular tissue showed lymphocytic infiltration around the bundles (Fig. 2). Three animals showed moderate reactions which were less severe than those just described yet more intense than those of the controls. Two animals showed only mild reactions, slightly more intense than the controls. One animal had a negative Arthus. This enhancement of the Arthus phenomenon must result from the increased antibody titer found in these adrenalectomized animals. The microscopic lesions which showed the massive infiltration of the dermis, and the necrosis of the uppermost epidermis, and particularly the infiltration about the muscle bundles just below the subcutaneous reaction were of interest because they suggested the lesion of dermatomyositis (Fig. 2).

Microscopic lesions: These lesions were graded according to the method described by us previously.[42] Four plus or markedly severe lesions were in those animals which showed extensive perivascular lesions in most of the tissues; 3+ or moderately severe lesions were in those animals who had slightly less extensive infiltration than the group with 4+ lesions; 2+ or mild lesions were those which showed perivascular infiltration in the heart and lungs alone; 1+ or a slight lesion were in those in which only slight lesions of perivascular infiltration occurred in one organ, usually the heart or the lungs. Microscopic sections of the 32 animals in this group were studied. Of these, 7 showed lesions of marked severity (4+) in at least 3 organs. These may be described as follows: the lesions in the lung (Fig. 3) appeared to be the most common and consisted of marked perivascular infiltration of the arteries and arterioles. The infiltrate consisted mainly of lymphocytes, but many plasma cells, neutrophils, and a few eosinophiles were also seen. The lesions in the pancreas of two animals were of particular interest, showing marked intravascular and perivascular infiltration with similar cells. The kidneys (Fig. 4) also presented lesions with periglomerular infiltration of lymphocytes, interstitial infiltration, and marked infiltration of the vessels in the medulocortical junction. The heart had not only lesions of mild myocardial infiltration but also severe perivascular lesions with infiltration of the previously described cells (Fig. 5). Of special note is the perivascular infiltration of the vessels in the portal canals which had not been noted before in any control animals. All 7 animals lived for 17 to 26 days after the initial injection of horse serum. Five animals in this first group of 32 rabbits showed moderately severe lesions (3+) which were slightly less extensive than in the group which had lesions previously described as markedly severe

Fig. 1.—Section through corium of positive Arthus phenomenon in an adrenalectomized rabbit. This shows fibrinoid degeneration, edema and infiltration of lymphocytes, plasma cells, and occasional eosinophiles.

Fig. 2.—Section through the muscle bundles below the corium in Arthus phenomenon in adrenalectomized rabbit. This shows infiltration of lymphocytes, plasma cells, and occasional eosinophiles.

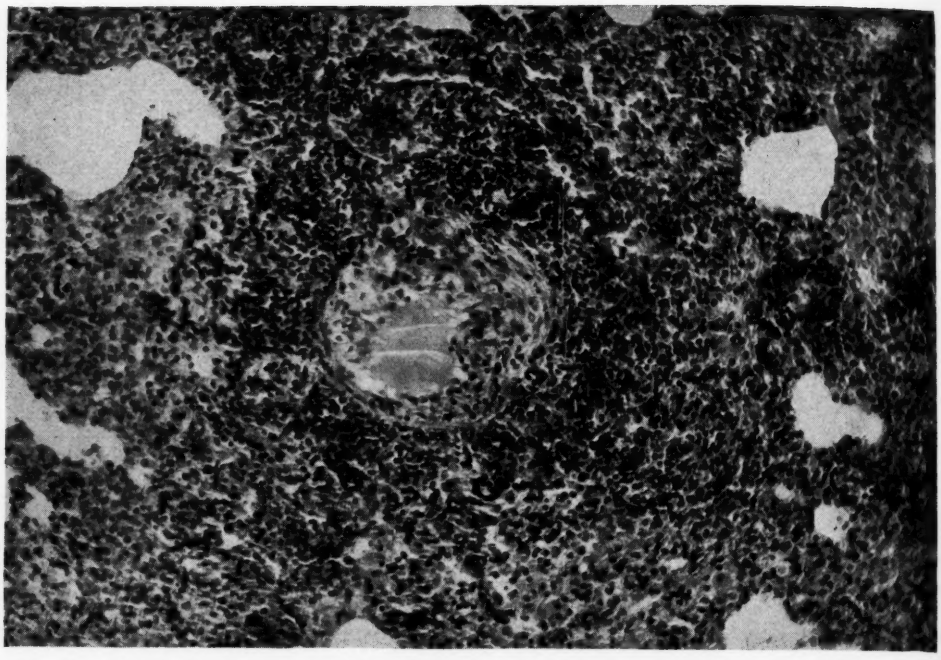

Fig. 3.—Section of lung from adrenalectomized and sensitized rabbit. Note perivascular cellular infiltration of pulmonary arteriole and capillary.

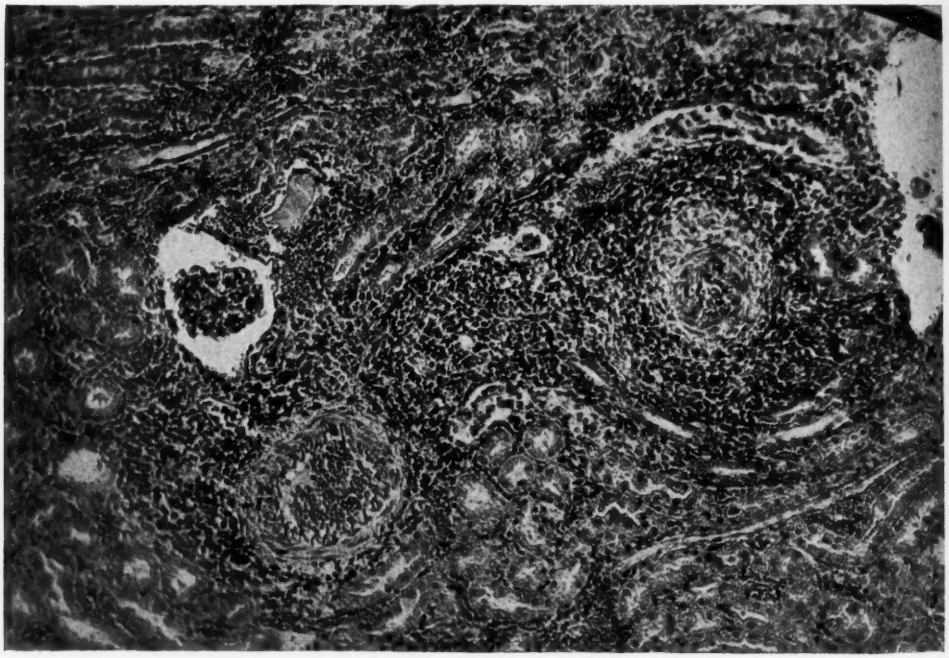

Fig. 4.—Section of kidney from adrenalectomized and sensitized animal. Note edema of media and adventitial infiltration of leucocytes about an arteriole. Note also the narrowed lumen as well as the cellular infiltration about the glomerulus and venule.

(4+). These animals lived for 13 to 30 days after initial sensitization. Six rabbits showed mild lesions (2+) and lived for 17 to 19 days. Four animals had slight lesions (1+) which were comparable to those of the control nonadrenalectomized group. These animals lived for 17 to 31 days and 2 of them died in anaphylaxis on the second injection of horse serum. Ten animals showed no lesions but these died from between the first and the thirteenth day, before the complete sensitization program could be carried out. One animal died in a convulsion during the initial administration of horse serum. Diffuse vascular reactions of the type described have been shown by Rich and Gregory,[3] as well as Criep and Mayer,[5] to be a manifestation of sensitization; hence it is considered an immunologic reaction depending upon antigen-antibody union. Since adrenalectomy increases antibody titer as just seen, it is logical to expect more extensive vascular lesions of this type to occur under such circumstances.

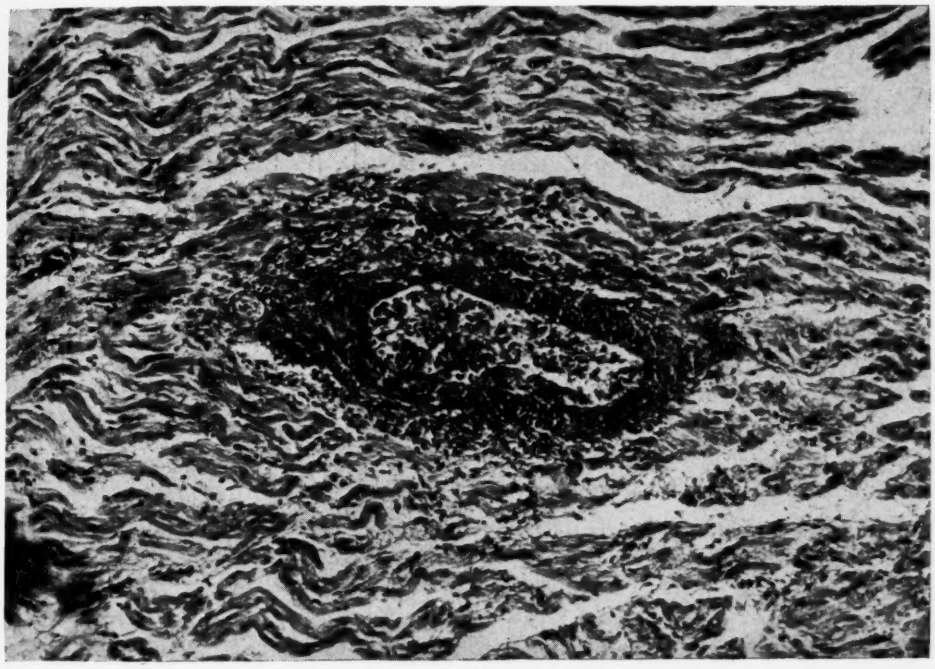

Fig. 5.—Section of heart muscle from adrenalectomized and sensitized rabbit. This shows a medium-sized blood vessel with infiltration of all layers by inflammatory cells.

Anaphylaxis: Of the 32 adrenalectomized animals in Group I sensitized by the technique referred to 7 died in anaphylactic shock after the second or third injection of antigen, and died in a convulsion during the initial sensitizing dose of horse serum. The latter was undoubtedly a toxic nonspecific reaction and not due to anaphylaxis. Anaphylactic shock was characterized by dyspnea, sneezing, choking and cyanosis, and retraction of the head in a state of exophthalmus. This indicated the increased vulnerability of the adrenalectomized animals to anaphylaxis, for in the control group of 10 animals sensitized by the same technique, but not adrenalectomized, none of the rabbits died in anaphylactic shock.

White blood counts and differential counts: A total of 12 animals out of this group were studied with white blood counts before and after adrenalectomy. These were done at least one week apart before sensitization with horse serum. The white blood cells varied from 2,000 to 17,850 per cubic millimeter before adrenalectomy. After adrenalectomy but before sensitization the range was from 6,950 to 18,700 per cubic millimeter. The average white count before adrenalectomy was 9,687, while after adrenalectomy it was 11,008, indicating only a slight leucocytosis. This may or may not have been due to the adrenalectomy. There was no appreciable difference in the differential counts before and after adrenalectomy except for slight neutropenia in 6 adrenalectomized animals. One animal showed a rise in the neutrophil count and 5 others showed little change in the neutrophils.

Total eosinophil counts: This was done in a total of 11 animals in this first group. Before adrenalectomy the counts varied from 42 to 87 per cubic millimeter. One count was as high as 203 per cubic millimeter, but this was in an animal with a high total white blood count of 17,850. After adrenalectomy, the counts varied from 25 to 137 per cubic millimeter. One count was 281; this appeared in an animal with a very elevated total white count of 18,700. The average counts were 77.8 per cubic millimeter and 81.3 per cubic millimeter before and after adrenalectomy, respectively. These determinations were made because of the recent interest in the eosinophil count as a reflection of adrenal cortical efficiency. It would seem, therefore, that adrenalectomy in the rabbit fails to produce a change in the total eosinophil count (Table II).

Comparison of body and splenic weights: The spleens were weighed without ligation of the hilum in all animals. The ratio of body weight to splenic weight was determined. This was done in 12 adrenalectomized animals and 11 unadrenalectomized animals, namely, 5 which received no horse serum or Doca, 3 which received horse serum and Doca, and 3 which received horse serum but no Doca. The body weights of the adrenalectomized animals varied from 1.8 to 3.2 kilograms and the splenic weights varied from 0.6 to 3.5 grams. An average of the total body weights was 2.3 kilograms and the average splenic weight was 1.77 gram in this group. In the control group of 11 animals the body weights varied from 2.3 to 4.5 kilograms or an average of 2.73 kilograms. The splenic weights varied from 1 to 5 grams or an average of 2.93 grams. It can be seen therefore that there is no appreciable increase in the weight of the spleen in the adrenalectomized group, although the organ appeared to be grossly larger at post mortem. This apparent contradiction is explained later in the paper.

All adrenalectomized animals showed gross enlargement of lymph nodes and thymus, while the control animals showed no such findings.

Group 2.—This group consisted of 2 rabbits. These animals were adrenalectomized and then sensitized with horse serum. No Doca was administered. The purpose of this part of the experiment was to demonstrate that such changes as were evident in the previous group were not due to the action of Doca. The animals showed the following: precipitin titers of 1-500 in one

and of 1-5000 in the other. The Arthus phenomenon developed to a very marked degree (4+) in both animals. Vascular lesions were evident to a moderate (2+) degree in one, and to a mild (1+) degree in the other. The first animal was sacrificed on the thirtieth day by air embolus, and the second died on the sixteenth day. Neither animal showed anaphylactic symptoms. Thus it is seen that the Doca in the dosage given played no role in Group 1 in causing a rise in precipitin titers, anaphylactic symptoms, or increasing the intensity of the vascular lesions.

Group 3.—This group consisted of 4 rabbits. These animals were adrenalectomized and maintained on Doca, but not sensitized. The purpose of this experiment was to see whether any of the changes observed in the sensitized animals could be attributed to adrenalectomy and Doca alone. As expected, none of these rabbits showed any of the phenomena of hypersensitiveness—no precipitins, Arthus phenomenon, or vascular lesions.

Group 4.—This group of 3 rabbits was adrenalectomized but not subjected to sensitization, and not given Doca. Adrenalectomy alone as expected produced none of the immunologic or pathologic phenomena of hypersensitiveness. They showed no precipitins, Arthus phenomenon or vascular lesions.

Group 5.—This group included 4 rabbits. They were not adrenalectomized, but were sensitized with horse serum and given the usual dosage of Doca. The purpose of this part of the experiment was to see whether Doca per se in any way changed the results obtained by sensitization, and also to represent a control group with which the results obtained in the adrenalectomized animals could be compared. Following are the results. Three animals had precipitin titers of 1-50, 1-500, and 1-500, respectively. The fourth died prematurely. Strong positive (4+) Arthus phenomena were obtained in all 4 rabbits. Microscopic lesions of diffuse vascular involvement were demonstrable to a moderate (2+) degree in 2 animals and only to a mild (1+) degree in the other 2. The first 2 died on the seventeenth and twenty-eighth days and the other 2 on the eighteenth and twenty-eighth days. Two of these animals died of anaphylaxis, one on the seventeenth day and one on the eighteenth day. It may be seen, therefore, that all the reactions of hypersensitiveness developed in this group; however, the precipitin titers were lower and the microscopic lesions were less advanced than in Group 1.

Group 6.—This group comprised 10 animals which were sensitized with horse serum but were not adrenalectomized and did not receive Doca.

The precipitin titers were as follows: one, 1-50; one, 1-100; two, 1-200; three, 1-500; one, 1-1,000, and two, 1-2,000. Only 1 of the 10 animals showed a marked positive Arthus phenomenon. In the others the reaction was only mild. The microscopic vascular lesions were completely absent in 2 animals; in 2, the lesions were moderately marked (3+); 1 had moderate lesions and in the rest the vascular changes were only mild. Hence, these changes, while present, were not nearly as diffuse or intensive as those observed in Group 1, where the animals were both adrenalectomized and sensitized. None of them showed anaphylactic shock.

TABLE II. COMPLETE BLOOD COUNT AND EOSINOPHIL COUNTS

	BEFORE ADRENALECTOMY								AFTER ADRENALECTOMY							
			DIFFERENTIAL					TOTAL EOSINOPHIL			DIFFERENTIAL					TOTAL EOSINOPHIL
ANIMAL	HB.	WBC	P	L	M	E	B	COUNT	HB.	WBC	P	L	M	E	B	COUNT
A38	12	10,400	36	52	9	3	0	42	13	9,650	19	72	1	8	0	47
A42	13	9,550	16	88	6	0	0	79	13.5	13,000	6	82	10	2	0	62
A45	10.5	14,000	41	56	3	0	0	85	13	11,600	28	66	2	3	1	137
A47	12	8,000	27	65	5	1	1	75	11	11,850	8	88	1	0	3	78
A49	11	10,600	20	76	3	3	0	59	11	11,400	13	80	3	2	2	78
A50	11.5	7,200	16	75	5	1	3	63	12	6,950	6	92	2	0	0	37
A51	13	16,500	20	76	1	3	0	87	13	18,700	41	52	5	1	1	281
A53	13	5,300	16	80	2	2	0	47	12	6,850	4	90	1	0	0	25
A54	13.5	5,600	15	75	5	2	3	69	11	9,300	22	77	0	1	0	75
A55	12	9,550	10	78	9	1	2	47	10	9,800	13	83	4	0	0	131
A56	—	17,850	16	81	2	1	0	203	13	10,600	21	70	3	5	1	44
A9	11	2,000	18	75	6	1	0	—	10	12,900	18	76	3	2	0	—

Group 7.—This group consisted of 2 animals which received only Doca. These rabbits were neither adrenalectomized nor sensitized. As was to be expected, neither of these rabbits showed precipitins, Arthus reaction, or vascular lesions.

TABLE III. SUMMARY OF GROUPS TREATED

GROUPS	NUMBER OF RABBITS	PROCEDURES EMPLOYED			RESULTS OBTAINED			
		ADRENALECTOMIZED	IMMUNIZED	DOCA	ANAPHYLAXIS	ARTHUS	PRECIPITIN	VASCULAR LESIONS
I	32	yes	yes	yes	yes	marked	high	marked
II	2	yes	yes	no	no	marked	high	marked
III	4	yes	no	yes	no	no	none	no
IV	3	yes	no	no	no	no	none	no
V	4	no	yes	yes	yes	marked	low	mild
VI	10	no	yes	no	yes	mild	low	mild
VII	2	no	no	yes	no	no	none	no

DISCUSSION

The question arises as to what manner the adrenalectomy enhances these various immunologic processes. The answer to this question in reality resolves itself in an attempt to state the relation of the adrenal cortex to these immunologic reactions. Such an attempt, in the light of our present knowledge, connotes oversimplification.

It is obvious from what preceded that the adrenal cortex controls the number of circulating lymphocytes. Our observations corroborate the findings of others; namely, that there is an inverse proportion between adrenal cortical activity and lymphoid mass,[44] although the adrenals are by no means the sole regulators of lymphoid tissue. Furthermore, Gordon and Katsh[45] pointed out that the adrenal cortex exercises a profound influence upon the phagocytic activity of the reticulo-endothelial cells. These cells in addition to certain protective functions are important in antibody formation.[46-48] There are indications that the lymphocyte is not only concerned with antibody transportation but may actually also be involved in antibody formation[12, 16, 17]; hence, hypertrophy of lymphoid tissues, which is in all likelihood the result of a mechanism secondary to the absence of the adrenal gland, could account for the presence of increased antibodies. At post-mortem examination, the spleens of the adrenalectomized animals appeared grossly enlarged; however, after considering their weight in relation to the total body weight no significant differences could be noted as compared with the unadrenalectomized animals. This may be attributed to the loss of blood after removal of the organ. The mesenteric lymph nodes of the adrenalectomized animals and the thymus were also enlarged grossly. The engorgement of the spleen is probably due to the absence of the adrenal glands since, as has been pointed out, adrenal cortical extract (A.C.E.) induces splenic discharge of lymphocytes into the circulation.[32, 49] This fact may also account for the failure of development of the expected lymphocytosis in the adrenalectomized rabbits. With the adrenals absent, the spleen presumably becomes enlarged and engorged with lymphocytes and splenic discharge fails to occur. Further indication of the relation of the adrenal cortex

to the lymphocyte is the fact that the administration of ACTH or cortisone in the intact animal produces a fall in the circulating lymphocytes,[16-19, 49] while ACTH does not produce lymphocytopenia in the adrenalectomized animal.[36, 50] Whether this lymphopenia is due to lympholysis or concentration of lymphocytes in the tissues[16] is not exactly known, although the body of evidence would seem to favor the latter view. And yet it would appear that while the lymphocyte may be considered as a source of antibody, there is serious doubt as to whether lymphocytic disintegration may yield a material increase in antibody titer.

One may speculate further into other possible mechanisms which increase the vulnerability of the adrenalectomized animal to sensitization.

A.C.E. depresses fibroblastic proliferation. The fibroblast is thought by many to be the basic cell of the reticulo-endothelial system. Therefore, removing the adrenals may possibly enhance mesenchymal or fibroblastic proliferation which in turn could account for a rise in antibody titers. Whether this is actually the case is not known. Moreover Bjorneboe, Fischel, and Stoerk have actually shown lower titers and decreased mononuclear cells and atrophic changes in the lymph nodes in sensitized animals receiving ACTH or cortisone.[58]

Another factor which may well play a role in the development of more severe diffuse vascular lesions in the sensitized animal after adrenalectomy is the potentiation of the spreading factor brought about by this procedure. It has been shown that the intracutaneous spreading of India ink is intensified by adrenalectomy and that the addition of hyaluronidase greatly enhances this effect.[51] Moreover inhibition of this spreading factor can be brought about by the addition of the corticosteroids, with or without hyaluronidase, in experimental animals.[52] Even in man, the increase in adrenocorticosteroid by epinephrine or ACTH decreases the activity of hyaluronidase in the skin.[53] Thus a direct relationship exists between the adrenal glands and this enzyme. It is possible that the extensive vascular lesions developed in our animals through sensitization were enhanced partially by this mechanism. In a similar manner one can suspect that other types of enzymatic action may also be a vital factor in this connection. There is evidence which indicates that the histamine level is increased and the histaminase level is decreased following adrenalectomy.[54-56] As a result of this lack of histaminase, the histamine level rises still higher following sensitization of the adrenalectomized animal and there results an increased susceptibility to the phenomena of anaphylaxis.[14, 15]

Is there an immunologic basis for the therapeutic effect of adrenal cortical steroids in allergic[29, 57] and collagen diseases?[37-41] A clear answer to this question is not easily forthcoming. It is not clear as to just how these therapeutic effects are brought about. Apparently, it is not because adrenal cortical steroids reduce antibody formation; however, it is possible that they may cause an interference with antigen-antibody union, or because the steroids affect the cell in such a way that it does not liberate the H-like substance[30, 46] following antigen-antibody reaction, or through some other, as yet undisclosed, chemical or enzymatic mechanism.

We have not touched in this discussion on the possibility that the presence of accessory adrenals in the rabbit may complicate our findings.

In spite of all the evidence suggested as to the possible role of the adrenal cortex in the immunologic phenomena of hypersensitiveness, and in spite of the findings herein referred to, the following should be pointed out: ACTH and cortisone have no effect on the Arthus phenomenon and on anaphylaxis; they questionably affect the tuberculin reaction; and, finally, in the intact animal they do not affect the antibody titer after sensitization. These and many other considerations make it impossible to be dogmatic in one's conclusions. The enhancement of sensitization following removal of the adrenals may mean that the adrenal cortex in the intact animal is concerned with immunologic processes, but it probably means equally that cortical steroids are essential to preserve certain functional capacities without the existence of which the tissue cells cannot be protected from the injurious effects of antigen-antibody union.

SUMMARY

It has been shown that adrenalectomy enhanced the immunologic reactions of hypersensitiveness. This included anaphylaxis, precipitin titer, and Arthus phenomenon. It also enhanced the development of experimental diffuse arteritis during sensitization.

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THE DEVELOPMENT OF TOLERANCE TO ANTIHISTAMINES*†

A Study of the Quantitative Inhibiting Capacity of Antihistamines on the Skin and Mucous Membrane Reaction to Histamine and Antigens

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THERE has been a growing feeling among some physicians that the prolonged use of antihistamines results in a development of tolerance or refractoriness to these drugs. Clinical observation alone, however, presents great difficulty in deciding whether tolerance has developed. This difficulty is based on 2 obvious facts: the variability of severity of allergic symptoms from period to period, and the tendency for each sufferer to establish a new standard of discomfort as time goes on. For example, a 75 per cent relief from an antihistamine may be gratifying to the patient at first, but several weeks later he may be evaluating the efficacy of the drug in terms of the remaining 25 per cent of symptoms. It was thought advisable, therefore, to investigate this problem by objective methods. The general scheme of the investigation was to note the amount of inhibition of the histamine and antigen reaction of the skin, nose, and eyes following an oral dose of an antihistamine, and to compare it at weekly intervals after continued use of the drug.

PROCEDURE

Initially we chose patients at random, using the skin to measure the nonspecific whealing and flare response to histamine or the whealing and flare response to an antigen. It soon became apparent that we were comparing 2 different procedures on different types of patients; the histamine response on patients with no demonstrable reaction to a specific antigen and skin reactions on patients in whom there was a specific antigenic response. Furthermore, we were assuming that the skin was a direct indicator of the shock organ response to both histamine and an antigen. Thus, it was decided to choose patients with a specific sensitivity and to test these patients with both the antigen and histamine, not only on the skin, but the shock organ as well. The type of patient that best fitted our criteria was the ragweed-sensitive individual with both nasal and ocular symptoms. In this group of patients titrations with graded concentrations of histamine and extract of ragweed were done on the volar aspects of the forearms and on the nasal and ocular membranes. Intracutaneous titrations were made because of the more uniform amount of histamine and antigen that could be delivered to the site of injection, 0.04 c.c. of each dilution being injected at each titration. A measured drop was instilled into the nose and also into the conjunctival sac.

The highest dilution producing a skin or mucous membrane response was used as the end point of each titration. Successive drops were instilled in the nose and conjunctival sac and after an interval of 10 minutes, if no reaction

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was apparent, the next stronger dilution was instilled, and so on until a reaction was apparent. The end point of the skin titration was considered to be that dilution that produced whealing and a flare response; the latter was found to be the most accurate. The wheal and flare were outlined on the skin by a ball point pen and the impression transferred by blotting with paper. Thus we had a direct record of the response for future comparison. The end point of the nasal mucous membrane titration was determined to be that dilution that produced both objective and subjective findings of edema of the mucous membrane and rhinorrhea, sneezing, or itching.

Since we were artificially producing a response in the mucous membranes and skin that naturally occurs in hypersensitive patients, the ability of an antihistamine to overcome or decrease this response was regarded as a measurement of its effectiveness to antagonize or counteract the insult. It follows, therefore, that if the reactions of the skin and ocular and nasal membranes of a patient are titrated before an antihistaminic drug is given and then the reactions of this same patient are titrated approximately one hour after an effective dose of an antihistamine, the diminution in the reactivity is a measurement of the ability of that antihistaminic drug to antagonize these responses. If this same patient is then placed on a maintenance dose of an antihistamine over a period of time and the same procedure repeated, the degree of inhibition of the skin and ocular and nasal membrane responses as compared to the inhibition in the original titration would be a measurement of the patient's ability to respond to the drug after prolonged use.

It was found that the usual therapeutic dose of most antihistamines often failed to produce clear-cut differences in the titrations; consequently, double the usual therapeutic dose was used in doing the titrations. All the patients were given the average clinical dose of an antihistamine 3 times daily as a maintenance dose.

All titrations were done on patients who had not eaten for at least 4 hours before the test was made. The repeat titrations were done an hour after an antihistamine was given, using the same area on the opposite arm and the opposite eye and nostril. In all titrations following maintenance therapy the drug was discontinued 24 to 36 hours or longer before the test was performed.

Fresh dilutions of extracts and histamine were prepared the day of the titrations. The variations as to temperature, strength of solutions, and physical and emotional state of the patient were minimized since we were not comparing absolute but only relative values, and each patient acted as his own control. We were simply comparing the reactions on comparable skin and mucous membrane sites using the same solutions before and after a dose of an antihistaminic drug. As Table I shows, the initial end points were somewhat variable on the same patients on different days. Each end point, however, was recorded as the base line for that patient for that particular titration, thus minimizing the number of variables.

The question of cross tolerance naturally arose. If a patient became tolerant to one antihistamine did he also evidence tolerance to other antihistamines? And, if cross tolerance exists, does it apply to all antihistamines, or does it

follow along lines of chemical relationship? We therefore did our initial titrations on different days, at least 48 hours between titrations, with 2 or 3 different drugs. The patient was then placed on a maintenance schedule of one specific antihistamine and after variable periods of maintenance was retitrated with histamine and ragweed extract before and after the same antihistamine as initially.

RESULTS

Skin Tests.—Table I shows the results of the intradermal histamine titrations in 20 subjects. These readings were taken before and one hour after an individual dose of an antihistamine and compared with the effect of the same antihistamine after the patient had taken the drug daily for a variable period. It will be noted in the cases of all the antihistaminic drugs that the tendency is to produce partial or complete tolerance to the drug in so far as it pertains to the ability of the drug to inhibit the histamine skin reaction. This development of tolerance usually was not present in a week, was noticeable after 2 weeks, and was well established at the end of 3 or 4 weeks. All but 2 subjects showed some tolerance to the drug after 14 or more days of its use. The drugs investigated, Benadryl, Neoantergan, Pyribenzamine, and Thephorin, showed no essential difference in this respect.

That this diminution of effectiveness of an antihistamine from its continued use is not confined to its action on introduced histamine is evident from the results noted in Table II. The figures in this table clearly indicate that the prolonged use of an antihistaminic drug decreases its ability to inhibit the skin reaction to an antigen, such as ragweed, and that this property is a function not of any one particular antihistamine, but at least several, and probably all. Of the 13 subjects in this series, 12 showed a development of tolerance.

Mucous Membrane Tests.—Tests made on the inhibiting effect of the antihistamines on the nasal and ophthalmic reactions to histamine and ragweed under conditions similar to the preceding also indicated a development of tolerance. In Table III are tabulated the results of the nasal titrations with histamine in 8 subjects. Of these, 5 showed definite diminution of action of the antihistamine after its use for 20 days. In the other 3 persons who took the drug for 14, 33, and 14 days, respectively, no decisive change was evident. The effect of the same procedure on nasal tests with ragweed showed evidence of the development of tolerance in 7 out of 10 subjects (Table IV).

The effect of prolonged use of an antihistamine on the ability of the same antihistamine to inhibit the conjunctival reaction to histamine is shown in Table V. It will be noted that in 6 out of 8 subjects the development of tolerance is apparent. Table VI shows the effects on conjunctival reactions to ragweed in 10 subjects. In 6 definite tolerance is shown, in 3 others tolerance is probable but not certain, and in 1 no tolerance was demonstrable.

It is probably significant that for the most part those patients who developed tolerance to an antihistamine as measured by histamine showed it also when tested with ragweed and those who showed it in skin testing also showed it by mucous membrane tests.

TABLE I. CHANGES IN INHIBITING EFFECT ON HISTAMINE SKIN REACTION AS A RESULT OF PROLONGED ANTIHISTAMINE ADMINISTRATION

PATIENT	HIGHEST DILUTION REACTING				PERIOD OF MAINTENANCE		
	BEFORE MAINTENANCE PERIOD OF ANTIHISTAMINE		AFTER MAINTENANCE PERIOD OF ANTIHISTAMINE				
	BEFORE TEST DOSE OF ANTIHISTAMINE	AFTER TEST DOSE OF ANTIHISTAMINE	BEFORE TEST DOSE OF ANTIHISTAMINE	AFTER TEST DOSE OF ANTIHISTAMINE	DRUG*	DOSE (MG. TID)	DAYS
T. W.	1:2,700,000	1: 900,000	1:2,700,000	1:2,700,000	Pyribenzamine	50	20
B. H.	1:8,100,000	1:2,700,000	1:2,700,000	1:2,700,000	Pyribenzamine	50	20
G. B.	1:8,100,000	1: 900,000 (–)	1:8,100,000 (–)	1:8,100,000	Pyribenzamine	50	20
J. S.	1:2,700,000	1: 900,000	1:2,700,000	1:2,700,000	Pyribenzamine	50	20
F. W.	1:2,700,000	1: 300,000	1: 900,000	1: 900,000	Pyribenzamine	50	20
J. M.	1:2,700,000	1: 300,000	1:2,700,000	1:2,700,000	Pyribenzamine	50	33
O. G.	1:2,700,000	1: 100,000	1:2,700,000	1: 900,000	Pyribenzamine	50	8
L. R.	1:2,700,000	1: 900,000	1:2,700,000	1:2,700,000	Pyribenzamine	50	20
			1:2,700,000	1:2,700,000			122
R. S.	1:8,100,000	1: 900,000	1:2,700,000	1: 900,000	Pyribenzamine	50	6
W. H.	1:8,100,000	1:2,700,000	1:8,100,000	1:2,700,000	Pyribenzamine	50	14
S. G.	1:8,100,000	1:2,700,000 (–)	1:8,100,000	1:8,100,000	Pyribenzamine	50	33
R. S.	1:2,700,000	1: 300,000	1:2,700,000 (–)	1: 900,000	Neoantergan	50	13
N. G.	1: 900,000	1: 100,000	1:2,700,000	1: 900,000	Neoantergan	50	20
G. B.	1:2,700,000	1: 300,000	1:2,700,000	1: 300,000	Neoantergan	50	26
S. B.	1:2,700,000	1: 300,000	1:2,700,000	1:2,700,000	Thephorin	25	20
M. S.	1:2,700,000	1: 900,000 (–)	1:2,700,000	1:2,700,000	Thephorin	50	21
L. W.	1:8,100,000	1: 900,000	1: 900,000	1: 900,000	Thephorin	50	13
J. B.	1:2,700,000	1: 900,000	1: 900,000	1: 900,000	Thephorin	50	26
J. M.	1:2,700,000	1: 900,000 (–)	1: 900,000	1: 900,000	Benadryl	50	20
M. B.	1:2,700,000	1: 300,000	1:2,700,000	1:2,700,000	Benadryl	50	20

*The same drug was used in titrations before and one hour after.
Titration doses used were as follows: Pyribenzamine 100 mg., Neoantergan, 100 mg., Thephorin, 50 mg., Benadryl, 100 mg.

TABLE II. CHANGES IN INHIBITING EFFECT ON RAGWEED SKIN REACTION AS A RESULT OF PROLONGED ANTIHISTAMINE ADMINISTRATION

PATIENT	HIGHEST DILUTION REACTING				PERIOD OF MAINTENANCE		
	BEFORE MAINTENANCE PERIOD OF ANTIHISTAMINE		AFTER MAINTENANCE PERIOD OF ANTIHISTAMINE				
	BEFORE TEST DOSE OF ANTIHISTAMINE	AFTER TEST DOSE OF ANTIHISTAMINE	BEFORE TEST DOSE OF ANTIHISTAMINE	AFTER TEST DOSE OF ANTIHISTAMINE	DRUG*	DOSE (MG. TID)	DAYS
T. W.	1:24,300,000	1:270,000	1:24,300,000	1:8,100,000	Pyribenzamine	50	20
B. H.	1:218,700,000	1:218,700,000	1:218,700,000	1:218,700,000	Pyribenzamine	50	20
G. B.	1:72,900,000	1:8,100,000	1:218,700,000	1:218,700,000	Pyribenzamine	50	20
J. S.	1:90,000	†	1:90,000	1:30,000	Pyribenzamine	50	20
F. W.	1:8,100,000	1:90,000	1:270,000	1:270,000	Pyribenzamine	50	20
J. M.	1:24,300,000	1:8,100,000 (–)	1:90,000	1:90,000	Pyribenzamine	50	20
E. K.	1:8,100,000	1:270,000 (–)	1:8,100,000	1:8,100,000	Pyribenzamine	50	13
C. N.	1:8,100,000	1:270,000 (–)	1:8,100,000	1:8,100,000	Pyribenzamine	50	21
F. S. (S)	1:218,700	1:24,300	1:2,700	1:2,700	Pyribenzamine	50	34
S. G. (S)	1:24,300	1:72,900	1:72,900	1:8,100	Pyribenzamine	50	33
A. G.	1:72,900,000	1:8,100,000	1:24,300,000	1:8,100,000	Neoantergan	50	13
C. N.	1:8,100,000	1:90,000	1:270,000	1:270,000	Neoantergan	50	20
I. H.	1:218,700,000	1:24,300,000	1:218,700,000	1:218,700,000	Thephorin	25	20

Titration doses used were as follows: Pyribenzamine 100 mg., Neoantergan 100 mg., Thephorin 50 mg.
(S) By scratch test.
*The same drug was used in titrations before and one hour after.
†Not done.

TABLE III. CHANGES IN INHIBITING EFFECT ON HISTAMINE NASAL REACTION AS A RESULT OF PROLONGED ANTIHISTAMINIC ADMINISTRATION

PATIENT	HIGHEST DILUTION REACTING: BEFORE MAINTENANCE PERIOD OF ANTIHISTAMINE — BEFORE TEST DOSE OF ANTIHISTAMINE	HIGHEST DILUTION REACTING: BEFORE MAINTENANCE PERIOD OF ANTIHISTAMINE — AFTER TEST DOSE OF ANTIHISTAMINE	HIGHEST DILUTION REACTING: AFTER MAINTENANCE PERIOD OF ANTIHISTAMINE — BEFORE TEST DOSE OF ANTIHISTAMINE	HIGHEST DILUTION REACTING: AFTER MAINTENANCE PERIOD OF ANTIHISTAMINE — AFTER TEST DOSE OF ANTIHISTAMINE	PERIOD OF MAINTENANCE — DRUG*	DOSE (MG. TID)	DAYS
F. W.	1:38,400	1:4,800	1:19,200	1:9,600	Pyribenzamine	50	20
J. S.	1:38,400	1:2,400	1:19,200	1:19,200	Pyribenzamine	50	20
J. M.	1:19,200	1:4,800	1:38,400	1:38,400	Pyribenzamine	50	20
G. B.	1:38,400	1:19,200	1:38,400	1:38,400	Pyribenzamine	50	20
B. H.	1:38,400	1:9,600	1:19,200	1:38,400	Pyribenzamine	50	20
W. H.	1:38,400	1:1,200	1:38,400	1:2,400	Pyribenzamine	50	14
S. G.	1:76,800	1:9,600	1:76,800	1:9,600	Pyribenzamine	50	33
K. T.	1:38,400	1:19,200	1:38,400	1:19,200	Pyribenzamine	50	14

*The same drug was used in titrations before and one hour after.

TABLE IV. CHANGES IN INHIBITING EFFECT ON RAGWEED NASAL REACTION AS A RESULT OF PROLONGED ANTIHISTAMINIC ADMINISTRATION

PATIENT	HIGHEST DILUTION REACTING: BEFORE MAINTENANCE PERIOD OF ANTIHISTAMINE — BEFORE TEST DOSE OF ANTIHISTAMINE	HIGHEST DILUTION REACTING: BEFORE MAINTENANCE PERIOD OF ANTIHISTAMINE — AFTER TEST DOSE OF ANTIHISTAMINE	HIGHEST DILUTION REACTING: AFTER MAINTENANCE PERIOD OF ANTIHISTAMINE — BEFORE TEST DOSE OF ANTIHISTAMINE	HIGHEST DILUTION REACTING: AFTER MAINTENANCE PERIOD OF ANTIHISTAMINE — AFTER TEST DOSE OF ANTIHISTAMINE	PERIOD OF MAINTENANCE — DRUG*	DOSE (MG. TID)	DAYS
F. W.	1:38,400	1:1,200	1:19,200	1:9,600	Pyribenzamine	50	20
J. S.	1:2,400	1:1,200	1:19,200	1:9,600	Pyribenzamine	50	20
J. M.	1:38,400	1:9,600	1:19,200	1:19,200	Pyribenzamine	50	20
G. B.	1:38,400	1:9,600	1:38,400	1:19,200	Pyribenzamine	50	20
B. G.	1:38,400	1:9,600	1:38,400	1:38,400	Pyribenzamine	50	20
A. T.	1:9,600	1:1,200	1:38,400	1:4,800	Pyribenzamine	50	20
K. T.	1:9,600	1:1,200	1:38,400	1:19,200	Pyribenzamine	50	15
F. S.	1:19,200	1:2,400	1:76,800	1:2,400	Pyribenzamine	50	34
M. M.	1:9,600	1:4,800	1:38,400	1:4,800	Pyribenzamine	50	32
S. G.	1:76,800	1:9,600	1:76,800	1:9,600	Pyribenzamine	50	32

*The same drug was used in titrations before and one hour after.

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TABLE V. CHANGES IN INHIBITING EFFECT ON HISTAMINE OPHTHALMIC REACTION AS A RESULT OF PROLONGED ANTIHISTAMINIC ADMINISTRATION

PATIENT	HIGHEST DILUTION REACTING				PERIOD OF MAINTENANCE		
	BEFORE MAINTENANCE PERIOD OF ANTIHISTAMINE		AFTER MAINTENANCE PERIOD OF ANTIHISTAMINE				
	BEFORE TEST DOSE OF ANTIHISTAMINE	AFTER TEST DOSE OF ANTIHISTAMINE	BEFORE TEST DOSE OF ANTIHISTAMINE	AFTER TEST DOSE OF ANTIHISTAMINE	DRUG*	DOSE (MG. TID)	DAYS
F. W.	1:19,200	1:2,400	1:38,400	1:19,200	Pyribenzamine	50	20
J. S.	1:19,200	1:4,800	1:38,400	1:38,400	Pyribenzamine	50	20
J. M.	1:38,400	1:4,800	1:38,400	1:38,400	Pyribenzamine	50	20
G. B.	1:38,400	1:9,600	1:38,400	1:38,400	Pyribenzamine	50	20
B. H.	1:38,400	1:9,600	1:38,400	1:38,400	Pyribenzamine	50	20
W. H.	1:19,200	1:1,200	1:9,600	1,1,200	Pyribenzamine	50	14
S. G.	1:38,400	1:4,800	1:38,400	1:4,800	Pyribenzamine	50	33
K. T.	1:38,400	1:9,600	1:19,200	1:19,600	Pyribenzamine	50	14

*The same drug was used in titrations before and one hour after.

TABLE VI. CHANGES IN INHIBITING EFFECT ON RAGWEED OPHTHALMIC REACTION AS A RESULT OF PROLONGED ANTIHISTAMINIC ADMINISTRATION

PATIENT	HIGHEST DILUTION REACTING				PERIOD OF MAINTENANCE		
	BEFORE MAINTENANCE PERIOD OF ANTIHISTAMINE		AFTER MAINTENANCE PERIOD OF ANTIHISTAMINE				
	BEFORE TEST DOSE OF ANTIHISTAMINE	AFTER TEST DOSE OF ANTIHISTAMINE	BEFORE TEST DOSE OF ANTIHISTAMINE	AFTER TEST DOSE OF ANTIHISTAMINE	DRUG	DOSE (MG. TID)	DAYS
F. W.	1:38,400	1:2,400	1:19,200	1:9,600	Pyribenzamine	50	20
J. S.	1:2,400	1:1,200	1:4,800	1:4,800	Pyribenzamine	50	20
J. M.	1:19,200	1:4,800	1:19,200	1:19,200	Pyribenzamine	50	20
G. B.	1:19,200	1:4,800	1:19,200	1:19,200	Pyribenzamine	50	20
B. H.	1:38,400	1:9,600	1:38,400	1:19,200	Pyribenzamine	50	20
A. T.	1:9,600	1:600	1:38,400	1:4,800	Pyribenzamine	50	20
K. T.	1:19,200	1:4,800	1:19,200	1:9,600	Pyribenzamine	50	15
F. S.	1:38,400	1:2,400	1:38,400	1:1,200	Pyribenzamine	50	34
M. M.	1:19,200	1:9,600	1:19,200	1:4,800	Pyribenzamine	50	32
S. G.	1:38,400	1:4,800	1:38,400	1:4,800	Pyribenzamine	50	14

TABLE VII. CROSS TOLERANCE

PATIENT	TEST MATERIAL	TITRATION BEFORE AND AFTER			MAINTENANCE DRUG	TITRATION BEFORE AND AFTER		
		Pyribenzamine (100 mg.)	Benadryl (100 mg.)	Neoantergan (100 mg.)	Pyribenzamine	Pribenzamine (100 mg.)	Benadryl (100 mg.)	Neoantergan (100 mg.)
1 E. R.	Histamine	4 – 3	4 – 2	5 – 5	50 mg. TID	4 – 4	4 – 4	5 – 5
2 R. S.	Histamine	5 – 3	4 – 2	4 – 4(–)	50 mg. TID	4 – 3(+)	4 – 4	4 – 4
3 O. G.	Histamine	4 – 1	3 – 2	4 – 3	50 mg. TID	4 – 3	3 – 3	3 – 3
4 C. N.	Ragweed	4 – 3	3 – 2(–)	4 – 2	50 mg. TID	4 – 4	2 – 2	3 – 3
5 E. K.	Ragweed	4 – 3(–)	5 – 3	3 – 3(–)	50 mg. TID	4 – 4	6 – 6	5 – 5
		Pyribenzamine (100 mg.)	Neoantergan (100 mg.)	Thephorin (50 mg.)	Neoantergan	Pribenzamine (100 mg.)	Pyribenzamine (50 mg.)	Neoantergan (100 mg.)
1 R. S.	Histamine	5 – 3	4 – 2		50 mg. TID	3 – 3 (–) 4 – 3	3 – 4	4(–) – 3
2 N. G.	Histamine	4 – 2(–)	3 – 1	4 – 3	50 mg. TID	4 – 2		4 – 2
3 G. B.	Histamine	4 – 2	4 – 3	4 – 2(+)	50 mg. TID	3 – 3 (–) 3 – 1	4 – 3	4 – 2 4 – 2
4 A. G.	Ragweed	4 – 2	6 – 4	5 – 4	50 mg. TID	5 – 4	7 – 7	
5 C. N.	Ragweed	4 – 3	4 – 2		50 mg. TID	3 – 1	3 – 3	3 – 2
		Histadyl (100 mg.)	Pyrrolazote (100 mg.)	Neoantergan (100 mg.)	Thephorin and Pyribenzamine	Thephorin (50 mg.)	Pyribenzamine (100 mg.)	Histadyl (100 mg.)
1 J. H.	Ragweed	5 – 4(+)	6 – 6(–)	7 – 5	Thephorin 25 mg. TID	7 – 7 7 – 7	6 – 4(–)	
2 S. B.	Histamine	4 – 2	4 – 5	4 – 3(–)	Pyribenzamine 50 mg. TID		4 – 4	4 – 3
		Pyribenzamine (100 mg.)	Benadryl (100 mg.)		Benadryl	Pribenzamine (100 mg.)	Benadryl (100 mg.)	
1 T. K.	Histamine	4 – 3	5 – 3		50 mg. TID	4 – 4		
2 J. N.	Histamine	3 – 2	4 – 3(–)		50 mg. TID	5 – 4	3 – 3	
3 M. B.	Histamine	4 – 3	4 – 2		50 mg. TID	5 – 4	4 – 4	
		Pyribenzamine (100 mg.)	Thephorin (50 mg.)		Thephorin	Pribenzamine (100 mg.)	Thephorin (50 mg.)	Benadryl (100 mg.)
1 M. S.	Histamine	4 – 3	4 – 3(–)		50 mg. TID	4 – 4	4 – 4	3 – 3
2 L. W.	Histamine	5 – 3	5 – 3		50 mg. TID	3 – 3	3 – 3	
3 J. B.	Histamine	3 – 2	4 – 3		50 mg. TID	2 – 1	3 – 3	

The reference numbers in the table represent the highest dilution giving a positive response intradermally. The dilutions used were as follows:

Histamine: 1 = 1:100,000; 2 = 1:300,000; 3 = 1:900,000; 4 = 1:2,700,000; 5 = 1:8,100,000

Ragweed: 1 = 30,000; 2 = 90,000; 3 = 270,000; 4 = 810,000; 5 = 2,430,000; 6 = 7,290,000; 7 = 21,870,000

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Pharmacologic Sensitivity After Discontinuance of the Antihistamine.—The natural question arises: How long does one remain refractory to an antihistamine after discontinuing its regular use? A complete answer to this question would require more extensive data than we were able to obtain at this time. However, our results in several instances with one procedure may furnish a clue to this phase of the problem. In 4 subjects who developed a tolerance to the effect of an antihistamine on the intracutaneous histamine reaction the following average results were obtained. Three days after discontinuance of the drug tolerance was still complete. At the end of 7 days partial inhibiting ability of the antihistamine began to appear and at the end of 14 days no further tolerance was apparent. It is not possible to say, of course, whether this course of events would apply to all persons.

Cross Tolerance.—The question of developing a tolerance to one antihistamine by taking another was studied with various combinations. Table VII summarizes our cross-tolerance studies, as determined by changes in the effect of antihistamines on the intradermal histamine or ragweed reaction. In the first part of the table it can be seen that 5 subjects who were given maintenance doses of Pyribenzamine, 50 mg. 3 times daily, developed a tolerance not only to Pyribenzamine, but also the Benadryl and Neoantergan. From the next section of the table it is apparent that a maintenance dose of Neoantergan, 50 mg., produced a suggestive but not striking tolerance to either Neoantergan or Pyribenzamine. It is very likely that the maintenance dose of 50 mg. of Neoantergan was insufficient for this purpose, since we had previously found Neoantergan to be a less potent antihistamine than Pyribenzamine.

From other sections of the table it becomes evident that the use of 25 to 50 mg. of Thephorin 3 times daily produces a tolerance to this drug. At the same time a tolerance is developed to Benadryl and to Pyribenzamine. The continued use of Benadryl, as shown in the same table, not only promotes a tolerance to Benadryl but also to Pyribenzamine. In one instance in which such a test was made some tolerance seemed to develop to Histadyl after maintenance doses of Pyribenzamine.

COMMENT

Although clinical experience with antihistaminic therapy over several years had made us suspicious of the possibility of the development of tolerance to these drugs, the incidence of such tolerance as shown by our objective tests came as a complete surprise. Also surprising was the rapidity of the onset of this tolerance. We emphasize the fact that, although a degree of refractoriness of these drugs from their repeated use can be shown by our methods, in most cases this amount of diminished effectiveness is apparently insufficient to interfere with therapeutic results. There is some indication that such tolerance cannot be shown as regularly by mucous membrane tests, the latter in this respect approaching more closely the clinical situation.

In extending our studies to various individual drugs and to various cross-tolerance tests there are some limitations. In the first place, much more effort and time would be required to study all drugs. In the second place, the weaker

drugs such as Neohetramine or Antistine do not lend themselves well to such a technique because with reasonable doses orally the inhibiting effect on skin or mucous membrane reactions is too slight or inconsistent.

The cross-tolerance studies, although limited in their extent, nevertheless suggest a definite interpretation. It will be noted that as far as chemical structure is concerned the compounds included ethylenediamine nuclei, thenyl radicals, ethers, and pyridindene. The chemistry is sufficiently varied so that tolerance on chemical grounds of the antihistamine is unlikely. We infer that the tolerance has to do with the common pharmacologic action of this series of drugs.

The report of Monash[1] on the inhibiting effect of an antihistamine on the histamine reaction produced iontophoretically in the skin is in general comparable to our findings with histamine skin titrations. Monash has claimed even more rapid development of tolerance than we were able to find with our methods.

SUMMARY

1. The majority of a series of patients showed evidence of the development of tolerance to antihistaminic drugs as shown by the failure or diminished effectiveness of the drugs to reduce the wheal and flare response to histamine and ragweed extract.

2. The time necessary to develop tolerance was between 7 and 20 days of maintenance on therapeutic doses of the antihistamine.

3. Tolerance developed to one antihistamine extended to others, even though the chemical relationship was not close.

4. Return of pharmacologic response to an antihistamine after discontinuance of the drug takes from 3 to 14 days.

5. We believe that, clinically, the development of tolerance is only relative. Even though our data would imply complete clinical tolerance in the greater part of our cases, most patients continue to derive symptomatic relief on prolonged antihistaminic therapy. The evidence presented does suggest, however, that patients on prolonged antihistamine therapy may develop clinical tolerance. When this is suspected the dose should be increased or the drug discontinued for a short perior (3 to 14 days) before resuming therapy.

REFERENCE

1. Monash, S.: Development of Refractory Condition of Skin Toward Anti-Histaminic Drugs After Anti-Histaminic Therapy as Determined by Histamine Iontophoresis, J. Invest. Dermat. **15**: 1, 1950.

THE EFFECT OF ACTH AND CORTISONE ON CERTAIN IMMUNOLOGIC MECHANISMS INCLUDING REVERSED ANAPHYLAXIS*†‡

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THE epoch-making studies of Hench, Kendall, and their co-workers have greatly stimulated interest in the effects of cortisone and adrenocorticotrophic hormone (ACTH) in a wide variety of diseases. Some of the most striking benefits with these hormones have been reported in allergic diseases of man. In order to determine the effectiveness and mode of action of these hormones in hypersensitive states, many investigators have studied their effect on the prevention or modification of anaphylaxis in animals.

Wolfram and Zwemer,[1] as well as Dragstedt, Mills, and Mead,[2] reported that adrenocortical extracts conferred some protection against anaphylactic shock in guinea pigs and dogs. On the other hand, Leger, Leith, and Rose,[3] Friedlaender and Friedlaender,[4] and Dworetzky, Code, and Higgins[5] found that ACTH offered no protection against anaphylactic shock in the guinea pig. In addition to this, the latter investigators[5] likewise observed cortisone ineffective against anaphylaxis in the guinea pig. In mice, however, according to Nelson, Fox, and Freeman,[6] cortisone gives some protection against fatal anaphylactic shock. The effects of these hormones in reversed anaphylaxis in guinea pigs have not, as yet, been reported. In both reversed and active anaphylaxis, the shock is due to the reaction between antigen and antibody. However, in active anaphylaxis the shock is produced by the injection of the antigen, while in reversed anaphylaxis, antibodies are injected which react with antigenic material normally present in certain tissues and cells of the animal. Moreover, active and reversed anaphylaxis of the guinea pig differ profoundly in symptomatology, pathology, and response to antihistaminic drugs (Arbesman, Neter, and Becker[7] and Hahn and Oberdorf.[8]

Therefore, it seemed of interest to determine the effects of ACTH and cortisone on reversed anaphylaxis in the guinea pig and to restudy their action on direct anaphylaxis. The action of cortisone on reversed Forssman anaphylaxis of the chick embryo was also studied. These investigations afforded an opportunity to observe and record the effect of ACTH on antibody formation in the rabbit. Studies of the ACTH effect on reagins in man were also carried out.

MATERIAL AND METHODS

Two preparations of ACTH were employed in these experiments: (1) the commercially available material (ACTH—Armour),‖ and (2) a product of

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‖Unless specified, the ACTH mentioned in the following experiments will be the ACTH now available commercially.

greater potency, Armour ACTH (79R).* Just prior to each experiment, the dried materials were carefully weighed and freshly dissolved in physiologic saline solution.

Cortisone (Cortone Acetate—Merck),† also commercially available, contained 25 mg. per c.c.

In each individual experiment untreated animals were used as controls. Both hormones were injected subcutaneously into female virgin guinea pigs weighing between 200 and 350 grams.

The challenging dose was given by intracardiac injection. All animals with fatal reactions were autopsied in order to exclude death due to trauma and to verify the gross pathologic findings characteristic of active or reversed anaphylaxis.

REVERSED FORSSMAN ANAPHYLAXIS IN THE GUINEA PIG

Forssman antibodies were prepared in rabbits by intravenous injections of 2.0 c.c. of a 2 per cent suspension of sheep cells 3 times weekly for 3 weeks. Following a rest period of 13 days, a second series of injections according to the same schedule was given for a period of 2 weeks. The animals were bled 10 days after the last injection. The sera were titrated for sheep cell agglutinins, and the 3 sera with the highest titers (1-1600, 1-3200, 1-3200) were used for the production of reversed anaphylaxis.

The minimal amount of each of these antisera (by intracardiac injection) necessary to produce fatal shock in the majority of guinea pigs was determined prior to each series of experiments. All experiments were not carried out at the same time; it was observed that upon prolonged storage in the deep freeze, the serum had lost some of its potency to produce reversed anaphylaxis. For each series of experiments the proper amount of serum was first determined, and it was found that in the later experiments the amount of antiserum had to be increased from 0.25 c.c. to 1 c.c.

Effects of ACTH.—ACTH (both types) was injected in doses of 0.1 mg. to 20.0 mg. in guinea pigs from 8 to 24 hours prior to the intracardiac injection of 0.25 to 1.0 c.c. of the Forssman antisera (antisheep cell [rabbit] sera). In the guinea pig, as will be discussed, ACTH and cortisone produce effects for several days after their administration. The results are shown in Table I.

The data presented indicate that ACTH in amounts of 0.5 to 2.0 mg. given 8 to 24 hours prior to the shocking dose had some protective effect. As compared to the control animals, ACTH lowered the immediate‡ death rate from 75 per cent to 36 per cent, and it prevented all signs of shock in 29 per cent as compared to only 2 per cent of the controls. In higher doses, ACTH (79R) 6.0 to 20.0 mg., no definite protective effect was noted. Likewise, a minimal dose of ACTH (79R) 0.1 mg. 24 hours prior to the shocking injection failed to give significant protection.

*Armour ACTH (79R) was kindly supplied by The Armour Laboratories, Chicago, Ill.

†Cortone Acetate was furnished through the courtesy of the Merck & Company, Inc., Rahway, N. J.

‡Only animals which died within one hour after the shocking intracardiac injection are included in this and all subsequent experiments.

The results of the experiments in which identical amounts (weight for weight) of the 2 preparations of ACTH were used suggest that lot 79R produced better protection than did the commercially available ACTH. For this comparison a total of 53 animals were available. Only 8 of 40 guinea pigs given the commercial ACTH in doses of 0.5 to 2.0 mg. 24 hours prior to the shocking injection was completely protected, whereas 6 of 13 guinea pigs injected with the same doses of ACTH (79R) had no signs of shock.

Effects of Cortisone.—Varying doses of cortisone (0.5 to 50 mg.) were injected into guinea pigs from 4 to 24 hours prior to the intracardiac injection of the Forssman antiserum just described. The effects of this substance on reversed anaphylaxis are shown in Table II. Cortisone, despite wide differences in the dosage schedule had little or no influence on the reversed Forssman anaphylactic shock.

TABLE I. EFFECT OF ACTH ON REVERSED FORSSMAN ANAPHYLAXIS IN GUINEA PIGS

ACTH		RESULTS			
		NUMBER OF ANIMALS			
AMOUNT (MG.)	TIME PRIOR TO SHOCKING INJECTION (HOURS)	TOTAL USED	FATAL SHOCK	SHOCK AND RECOVERY	NO SHOCK
(None) CONTROLS		48	36	11	1
0.1*	24	10	8	2	0
0.5*	24	9	4	1	4
0.5	24	35	12	15	8
0.5	8	5	2	1	2
2.0*	24	4	2	0	2
2.0	24	5	1	4	0
2.0*	8	3	1	0	2
6.0*	24	6	6	0	0
12.0*	24	6	6	0	0
20.0*	24	2	2	0	0
20.0*	8	4	2	2	0

*Preparation 79R; all others are the commercially available preparation.

REVERSED ANAPHYLAXIS IN GUINEA PIGS

Rabbits were injected intravenously with 2.0 c.c. of normal guinea pig serum 3 times weekly for 3 weeks. Ten days after the last injection the animals were bled and the precipitin titers of the rabbit antisera determined. The antisera with the highest titers (1-20,000 and 1-40,000) were used. The minimal amount of these antisera (0.5 to 1.0 c.c.), which, when injected into the hearts of the guinea pigs, produced fatal shock in the majority of animals was determined prior to each series of experiments.

Effects of ACTH.—ACTH was injected into guinea pigs in doses of 6 mg. or 10 mg. The time interval between these injections and the intracardiac injection of the antiguinea pig serum (rabbit serum) varied from 1 to 4 hours. The data of this experiment are shown in Table III and indicate that ACTH affords some protection. While 13 (85 per cent) of the 20 control animals died in acute shock, only 6 (30 per cent) of 20 ACTH-treated animals had fatal reactions. Likewise, only one (5 per cent) of the 20 controls failed to show any signs of reversed shock, but 6 (30 per cent) of the treated animals exhibited no evidence of shock.

TABLE II. EFFECT OF CORTISONE ON REVERSED FORSSMAN ANAPHYLAXIS IN GUINEA PIGS

CORTISONE		RESULTS			
		NUMBER OF ANIMALS			
AMOUNT (MG.)	TIME PRIOR TO SHOCKING INJECTION (HOURS)	TOTAL USED	FATAL SHOCK	SHOCK AND RECOVERY	NO SHOCK
(None) CONTROLS		34	27	6	1
0.5	4	2	2	0	0
	7	1	1	0	0
5	4	2	2	0	0
	7	6	5	1	0
	24	4	4	0	0
12.5	24	4	3	0	1
25	4	2	2	0	0
25	7	2	2	0	0
25	24	18	13	3	2
50	8	4	4	0	0
50	24	4	2	0	2

Effects of Cortisone.—Cortisone, in doses of 25 mg. or 50 mg., was injected into guinea pigs one hour prior to the shocking dose of anti-guinea pig serum (rabbit serum). The results in Table IV show that cortisone, too, protects to a certain degree against reversed anaphylactic shock. Eight of 21 control animals had acute fatal shock, while only 1 of the 20 cortisone-treated animals died. Likewise, all 21 untreated animals had severe signs of shock, but 5 of the treated guinea pigs were completely unaffected by the shocking injection.

TABLE III. EFFECT OF ACTH ON REVERSED ANAPHYLAXIS IN GUINEA PIGS

ACTH		RESULTS			
		NUMBER OF ANIMALS			
AMOUNT (MG.)	TIME PRIOR TO SHOCKING INJECTION (HOURS)	TOTAL USED	FATAL SHOCK	SHOCK AND RECOVERY	NO SHOCK
(None) CONTROLS		20	13	6	1
6	1	8	4	0	4
6	4	7	1	4	2
10	4	5	1	4	0

DIRECT ANAPHYLAXIS IN GUINEA PIGS

Active Anaphylaxis.—Forty-nine guinea pigs were sensitized by an intracardiac injection of rabbit serum (0.5 c.c. to 1.0 c.c.). Eleven to 18 days later a challenging dose of 1.0 c.c. of normal rabbit serum was injected into the hearts of these animals.

TABLE IV. EFFECT OF CORTISONE ON REVERSED ANAPHYLAXIS IN GUINEA PIGS

CORTISONE		RESULTS			
		NUMBER OF ANIMALS			
AMOUNT (MG.)	TIME PRIOR TO SHOCKING INJECTION (HOURS)	TOTAL USED	FATAL SHOCK	SHOCK AND RECOVERY	NO SHOCK
(None) CONTROLS	–	21	8	13	0
25	1	12	1	6	5
50	1	8	0	8	0

Effect of ACTH: The day after the sensitizing injection 4 of these guinea pigs were given 2.0 mg. of ACTH (79R) daily for 11 days until the challenging injection. Ten other sensitized animals of this group received 6 mg. of ACTH, 34 hours, 24 hours, and 8 hours (total ACTH, 18 mg.) prior to the shocking injection. Sixteen guinea pigs sensitized in like manner were not treated, and served as controls. As can be seen from Table V, ACTH in the dosage and time intervals employed failed to protect the guinea pigs from active anaphylactic shock, and, also, did not prevent sensitization in the 4 guinea pigs used.

TABLE V. EFFECT OF ACTH ON ACTIVE ANAPHYLAXIS IN GUINEA PIGS

ACTH		RESULTS			
		NUMBER OF ANIMALS			
AMOUNT	TIME PRIOR TO SHOCKING INJECTION	TOTAL USED	FATAL SHOCK	SHOCK AND RECOVERY	NO SHOCK
(None) CONTROLS		16	5	7	4
2 mg. × 11	*	4	1	3	0
6 mg. × 3	*	10	3	6	1

*For schedule see text.

Effect of Cortisone: Cortisone (25 mg.) was given to 5 guinea pigs, one hour before, and to an additional 4 guinea pigs, 24 hours before the challenging injection of 1 c.c. of normal rabbit serum. Ten sensitized animals did not receive this hormone and served as controls. The results are presented in Table VI. It is evident that cortisone, under the preceding experimental conditions, did not alter the outcome of active anaphylaxis.

TABLE VI. EFFECT OF CORTISONE ON ACTIVE ANAPHYLAXIS IN GUINEA PIGS

CORTISONE		RESULTS			
		NUMBER OF ANIMALS			
AMOUNT (MG.)	TIME PRIOR TO SHOCKING INJECTION (HOURS)	TOTAL USED	FATAL SHOCK	SHOCK AND RECOVERY	NO SHOCK
(None) CONTROLS		10	3	7	0
25	1	5	3	2	0
25	24	4	1	3	0

Passive Anaphylaxis.—Thirty-eight guinea pigs were injected intraperitoneally with a highly potent antihuman serum (rabbit serum) (titer 1-50,000). Twenty-four to 48 hours later, they were shocked by the intracardiac injection of 0.25 c.c. to 1.0 c.c. of normal human serum.

Effect of ACTH: Four hours prior to the sensitizing dose, corresponding to 28 hours prior to the shocking dose, 0.5 mg. of ACTH (79R) was injected into 6 animals. As can be seen from Table VII, no protection was afforded from passive anaphylactic shock, nor did ACTH prevent passive sensitization.

Effect of Cortisone: The effects of various amounts of cortisone (0.5 mg. to 25 mg.) 6 hours prior to the shocking dose is indicated in Table VIII. Here,

again, one notices the lack of protective action of this hormone in passive anaphylaxis, inasmuch as all animals developed severe symptoms of shock. The data suggest that 5 mg. of cortisone given 6 hours prior to the shocking dose may have somewhat lowered the death rate.

TABLE VII. EFFECT OF ACTH ON PASSIVE ANAPHYLAXIS IN GUINEA PIGS

ACTH		RESULTS			
		NUMBER OF ANIMALS			
AMOUNT	TIME PRIOR TO SHOCKING INJECTION (HOURS)	TOTAL USED	FATAL SHOCK	SHOCK AND RECOVERY	NO SHOCK
(None) CONTROLS		12	10	2	0
0.5*	28	6	6	0	0

*79R.

EFFECT OF CORTISONE ON REVERSED FORSSMAN ANAPHYLAXIS OF THE CHICK EMBRYO

In the 3-day-old chick embryo, a phenomenon can be produced which closely resembles reversed Forssman anaphylaxis (Baumann and Witebsky[9]). It is due to the action of normal or immune Forssman antibodies on Forssman antigen normally present in the chick embryo. It manifests itself by vascular contractions and death of the embryo. Cortisone alone, in doses of 0.025 mg. to 7.5 mg., had no direct noticeable effect, nor did it prevent the shock caused by Forssman antibodies (normal human serum) when administered in the preceding dosage, either 1 to 5 hours prior to, or concomitant with the administration of these antibodies.

EFFECT OF ACTH ON ANTIBODY FORMATION

Precipitins.—Seven rabbits were given intravenous injections of 2.0 c.c. of normal guinea pig serum 3 times weekly for 3 weeks. Of this group of animals, 3 were given 12.5 mg. of ACTH 4 hours prior to each sensitizing injection of

TABLE VIII. EFFECT OF CORTISONE ON PASSIVE ANAPHYLAXIS IN GUINEA PIGS

CORTISONE		RESULTS			
		NUMBER OF ANIMALS			
AMOUNT (MG.)	TIME PRIOR TO SHOCKING INJECTION (HOURS)	TOTAL USED	FATAL SHOCK	SHOCK AND RECOVERY	NO SHOCK
(None) CONTROLS		12	10	2	0
0.5	6	4	3	1	0
5	6	12	7	5	0
25	6	4	3	1	0

antigen. Following the last injection of guinea pig serum, there was a rest period of 10 days prior to the final bleeding. During this period, 2 animals of the control group (no ACTH during the immunization period) were injected with 3 mg. of ACTH 4 times daily for 8 days. Precipitin titers were performed on blood obtained from all animals on the twenty-ninth and thirty-third day

of the experiment, and are incorporated in Table IX. As can be seen from this table, ACTH, in the amounts and time intervals given, had no effect on either increasing or decreasing the antibody (precipitin) titer.

Agglutinins.—Twelve rabbits were injected with 2.0 c.c. of a 2 per cent suspension of sheep cells 3 times weekly for 3 weeks. Six of these rabbits received 12.5 mg. of ACTH 4 hours prior to each sensitizing injection (Groups 3 and 4). The remaining 6 served as controls (Groups 1 and 2). During the first rest period, (twenty-first to thirty-fifth day after first injection of antigen) 3 of the ACTH-treated animals (Group 4) and 3 of the untreated rabbits (Group 2) were given 3 mg. of ACTH 4 times daily for 15 days. Trial bleedings on all animals were done on the twenty-fifth, twenty-ninth, and thirty-third day. The latter 2 bleedings corresponded to the eighth and thirteenth day of this series of ACTH treatment (Table X). In view of the low titers obtained in all animals, they were reimmunized with 2.0 c.c. of a 2 per cent suspension of sheep cells 3 times weekly for another 2 weeks. Ten and 21 days after this second course of sensitization (corresponding to the fifty-sixth and sixty-seventh day from the start of this experiment), the agglutinin titers were again determined (Table X). Then, in order to investigate whether or not

TABLE IX. EFFECT OF ACTH ON PRECIPITIN (ANTINORMAL GUINEA PIG SERUM ANTIBODIES) FORMATION IN THE RABBIT

DAY AFTER FIRST ANTIGEN INJECTION	PRECIPITATION TITER × 10.000						
	GROUP 1 CONTROLS		GROUP 2 ACTH DURING SENSITIZATION*			GROUP 3 ACTH DURING REST PERIOD	
RABBIT NO.	1	2	3	4	5	6	7
29th	1:4	1:4	1:4	1:4	1:4	1:4	1:2
33rd	1:8	1:8	1:2	1:2	1:2	1:5	1:1

*For schedule see text.

ACTH may produce a prompt, sudden rise in antibodies, 3 of these rabbits were given a single injection of 30 mg. of ACTH, and 2 others serving as controls received a like volume of saline solution. The antibody titers of these 5 animals were determined 9 hours after the ACTH treatment (Table X).

TABLE X. EFFECT OF ACTH ON AGGLUTININ (SHEEP CELLS) FORMATION IN THE RABBIT

DAY AFTER FIRST ANTIGEN INJECTION	GROUP 1 NO ACTH			GROUP 2 ACTH DURING FIRST REST PERIOD			GROUP 3 ACTH DURING FIRST SENSITIZATION			GROUP 4 ACTH DURING FIRST SENSITIZATION AND REST PERIOD		
RABBIT NO.	1	2	3	4	5	6	7	8	9	10	11	12
	AGGLUTININ TITER × 100											
25th	1:2	1:2	1:2	1:2	1:1	1:1	1:1	1:2	1:3	1:2	1:1	1:1
29th	1:2	1:4	1:2	1:1	1:2	1:2	1:1	1:2	1:2	1:2	1:1	1:2
33rd	<1:1	<1:1	1:1	1:0.4	<1:1	<1:1	1:0.1	<1:1	<1:1	1:0.8	<1:1	<1:1
56th	+	1:4	1:2	1:4	1:4	1:8	+	+	1:32	1:32	1:4	1:8
67th			1:4	1:4	1:16							1:4
69th			NaCl	ACTH	ACTH						ACTH	NaCl
69th			1:8	1:8	1:46						1:2	1:4

+Died.

All the data obtained indicate that ACTH in the dosages just given failed to affect the demonstrable agglutinin titers, either during the sensitization period or during the rest period, nor did it produce a booster effect in sensitized animals.

EFFECT OF ACTH ON REAGIN TITER IN MAN

Another immune mechanism studied was that of the reagin titer in man. Blood was taken from each of 3 ragweed-sensitive patients prior to a course of ACTH therapy. The sera were frozen and stored until ready for use. Each of these patients received between 600 mg. and 800 mg. of ACTH over a period of 7 to 10 days. Four hours after the last injection of ACTH, each patient was again bled, and the sera were frozen and stored. Serial titrations (two-fold) of each of the pre- and post- ACTH-treatment sera were performed on each of 2 nonallergic recipients. No measurable difference in the reaginic titer could be noted.

DISCUSSION

The purpose of this investigation was to study the effects of ACTH and cortisone on various types of immune mechanisms. One of the chief problems confronting the investigator in the use of these hormones in animals is the establishment of effective dosage schedules. The eosinophile response of guinea pigs to injections of ACTH and cortisone was previously studied by Dworetzky and associates.[5] Absolute eosinophile counts were performed on 16 guinea pigs before and from 1 hour to 7 days after the injection of 0.01 mg. to 20.0 mg. of ACTH. It was found that ACTH in amounts of 0.5 to 20.0 mg. reduced the eosinophile count within 5 hours, and for as long as 7 days. Cortisone in amounts of 5 mg. to 50 mg. was given to 8 guinea pigs for studies on eosinophilic response. These cells dropped within 4 hours after these injections and remained low during the observation period of 96 hours. Physiologic saline solution, alone, when injected into 8 guinea pigs, did not produce an eosinopenia. These data confirm the observations of Dworetzky et al.[5] Hence, in our later experiments (Forssman and passive anaphylaxis), dosage schedules according to the eosinophilic response were employed.

Unfortunately, this same technique cannot be applied to rabbits, because of the number of pseudo eosinophiles present in the circulating blood. However, an attempt was made to determine the blood glucose response to ACTH in rabbits as a guide to dosage determination. This approach was soon discarded, as the results were neither regular nor reproducible by the methods and techniques employed. Therefore, the dosages of ACTH used in the rabbits were arbitrarily chosen from the experiences of previous investigators.

The importance of dosage is apparent from the preceding experiments on reversed and Forssman anaphylaxis. ACTH (0.5 mg. to 2.0 mg.) given 24 hours prior to the shocking dose afforded a greater degree of protection than did larger doses (6 mg. to 20.0 mg.). However, when these same larger doses of ACTH were administered only a short time (1 to 4 hours) prior to the shocking injection, some protection was manifested. This may be due to the

fact that at the time of the shocking injection the effects of the total amount of hormone injected were not yet at maximum. On the other hand, the ineffectiveness of the large dose of ACTH at the optimal (according to the eosinophile response) time (24 hours) might be the result of adrenal exhaustion.

Of the 2 preparations of ACTH used in these studies, it appears that, weight for weight, preparation 79R was somewhat more protective in reversed and Forssman anaphylaxis.

Although ACTH and cortisone prevented acute fatal reversed shock in a fair percentage of animals, many of them had delayed deaths from 1 to 10 days later. This could conceivably be due to the pathologic involvement of the central nervous system. Jervis[10] reported that such damage does occur when sub-lethal doses of Forssman antibody are injected. These hormones, by preventing death, may have produced such a delayed response. Further studies are now in progress in an attempt to explain this problem.

The failure of ACTH or cortisone to prevent active or passive anaphylaxis confirms the work of previous investigators. This is further evidence that, although both reversed and direct anaphylaxis are the result of an antigen-antibody reaction, their mechanism of action differs considerably. The antihistaminics protect against active anaphylaxis in the guinea pig. The hormones studied offer some protection against reversed shock, but little or none against direct anaphylaxis. Likewise, in the mouse, antihistaminics are ineffective, but ACTH is somewhat effective in preventing anaphylactic shock. In the chick embryo, on the other hand, neither the antihistaminics (Neter[11]) nor cortisone prevented the reversed Forssman reaction.

The beneficial effects of ACTH and cortisone in allergic disorders of man and in acute and reversed anaphylaxis of different animals may be operative through influences on: (1) the antibody formation, (2) antibody fixation to tissues, (3) tissue reaction to the antigen-antibody union, and (4) the reactivity of the cells of the various shock organs.

No effect on antibody production in the rabbits treated according to the schedules described was noted. The discrepancies of opinion in the literature[12, 13] regarding the effects of ACTH on antibody formation may be due to many factors, for example, the types of antigens used, the amounts, potency, and purity of the hormones, and the wide difference in dosage schedules.

ACTH given 4 hours prior to a passively sensitizing dose of antibody did not prevent sensitization. This indicates that ACTH did not prevent the fixation of the sensitizing antibody at the site of the shock organ. Likewise, ACTH given daily during the course of active sensitization did not influence the development of the state of hypersensitivity. The failure of ACTH to effect the development of sensitizing antibodies is in accord with its failure to alter the production of precipitins and agglutinins as herein reported.

Although ACTH was given 3 times weekly to rabbits for a period of 3 weeks, antibodies against this hormone were not demonstrated by precipitin tests in the sera of these animals.

SUMMARY AND CONCLUSIONS

1. ACTH and cortisone in the scheduled dosages employed does protect a certain percentage of animals from immediate fatal reversed and Forssman anaphylactic shock.

2. These substances afford little or no protection against direct active or passive anaphylaxis in the guinea pig.

3. Cortisone does not prevent or alter the reversed Forssman anaphylaxis in the chick embryo.

4. The antibody formation in rabbits and the development of active and passive sensitization in the guinea pig are not altered by the amounts of ACTH given.

5. The reaginic titer in sera of man was unaltered by ACTH therapy.

6. The mode of action of these hormones in reversed and Forssman anaphylaxis in guinea pigs remains to be elucidated.

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STUDIES IN BACTERIAL ALLERGY

V. Persistence of Desensitization, With Bacterial Haptens, of Guinea Pigs Sensitized Passively With Heterologous Serum*†

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DESENSITIZATION of passively sensitized guinea pigs with bacterial haptens has been reported.[1] The duration of this desensitization has not been studied. Desensitization of actively sensitized guinea pigs with whole antigens[2] and desensitization of the passively sensitized guinea pig uterus with a bacterial hapten[3] are transient. Passive sensitization with heterologous serum is transient.[4, 5] Passive sensitization with homologous serum is lasting.[6]

These experiments were planned to study the duration of bacterial hapten desensitization of guinea pigs after passive sensitization with heterologous serum.

METHODS

Guinea pigs were used as experimental animals. Capsular pneumococcal polysaccharides,‡ Types I, II, and IV, were used and are referred to as haptens. Corresponding types of refined antipneumococcal rabbit serum§ were used as heterologous passively sensitizing antibodies. Types I and II antisera contained 4,000 units and type IV 6,000 units per milliliter.

Passive sensitization was produced by intra-abdominal injections of 0.2 to 0.5 ml. of the antiserum.

Desensitization was done by intra-abdominal injections of specific polysaccharides. Desensitization was proved by an intravenus challenging dose of specific polysaccharide in all but one experiment. Details are given in the tables. In preliminary experiments in 5 animals, subcutaneous injections of 5.5 mg. of polysaccharide in divided doses in 2 days did not desensitize. This method was not studied further.

Challenging doses of polysaccharide to test the presence of hypersensitiveness were given intravenously. Preliminary experiments had shown that 1.0 mg. would elicit anaphylactic shocks quite regularly.

Experiment I shows the approximate duration of passive sensitization. Details are given in Table I. The data suggest that anaphylaxis can be produced regularly up to 9 days under the conditions of this experiment. Anaphylaxis was produced irregularly after 9 days and not at all after 13 days. A more accurate determination of the duration of passive sensitization would require standardization of the variables: types, dose, and strength of sensitizing antiserum; minimal shocking doses of polysaccharide for each type of antiserum; age, weight, and diet of the guinea pig. A more accurate determination was not thought to be germane to the current problem.

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TABLE I. DURATION OF PASSIVE SENSITIZATION

GUINEA PIG NUMBER	SENSITIZING ANTISERUM (I.P.) DOSE (ML.)	TYPE	INTERVAL (DAYS)	CHALLENGE SPECIFIC CHO (I.V.) (MG.)	REACTION
1	0.25	2	2	2.0	AD
2	0.25	4	2	2.0	AD
3	0.5	2	7	1.25	AD
4	0.5	2	7	1.25	AD
5	0.5	2	8	1.25	2+
6	0.5	2	9	1.25	2+
7	0.2	2	9	1.25	AD
8 & 9	0.25	2	10	2.0	O
10 & 11	0.25	4	10	2.0	O
12	0.5	2	11	1.25	O
13	0.5	2	11	1.25	2+
14	0.5	2	11	1.25	AD
15, 16	0.2	1	14	1.25	O
17, 18					
19, 20	0.2	1	16	1.25	O
21, 22					

I.P. = intra-abdominally.
CHO = pneumococcal polysaccharide.
2+ = marked respiratory difficulty, recovered.
AD = anaphylactic death.

Experiment II shows that bacterial haptens will desensitize specifically and that the desensitization is lasting. Details are given in Table II. Note that 1.25 mg. of specific polysaccharide intra-abdominally desensitized 2 guinea pigs to the extent that they did not react to an intravenous challenging dose the next day. On the other hand, 1.0 mg. of polysaccharide intra-abdominally desensitized only 6 of the remaining 16 animals.

Note, too, that the 2 animals desensitized by 1.25 mg. of hapten initially and 4 animals which were not desensitized by 1.0 mg. (but survived the reaction to the intravenous test of desensitization) remained desensitized when challenged with hapten 7 days later. The other animals were not challenged after the initial tests of desensitization.

Experiment III shows that nonspecific haptens do not prevent later specific anaphylactic reactions. Details are given in Table III. Three things should be noted. First, there were no reactions to the intra-abdominal nor to the intravenous injections of the nonspecific ploysaccharide. Second, the nonspecific polysaccharide intra-abdominal and intravenous doses were the same as the specific doses which produced desensitization in Table II. Finally, anaphylaxis was not prevented when the animals were challenged with specific polysaccharide 7 days later. The intensity of the reactions was somewhat less than anticipated. No effort was made to explain this.

Experiment IV shows 2 things in guinea pigs passively sensitized with 2 types of antiserum: first, that hapten desensitization is lasting and specific. Second, desensitization with one specific hapten does not prevent anaphylactic reaction to intravenous injection of the other specific hapten. Details are given in Table IV. Note that 3 intra-abdominal desensitizing doses of specific polysaccharide were used instead of the intra-abdominal and intravenous desensitizing doses shown in Table II. Other experiments not given in detail had shown

TABLE II. DESENSITIZATION STUDIES

GUINEA PIG NUMBER	SENSITIZING ANTISERUM (I.P.) DOSE (ML.)	TYPE	INTERVAL (DAYS)	DESENSITIZATION SPECIFIC CHO (I.P.) (MG.)	INTERVAL (DAYS)	I.V. TEST OF DESENITIZATION WITH SPECIFIC CHO (I.V.) (MG.)	REACTION	INTERVAL (DAYS)	CHALLENGE SPECIFIC CHO (I.V.) (MG.)	REACTION
1	0.2	2	1	1.25	1	1.25	O	7	1.25	O
2	0.2	2	1	1.25	1	1.25	O	7	1.25	O
3	0.2	2	1	1.0	1	1.25	AD	–	–	–
4	0.4	2	1	1.0	1	1.25	3+	7	1.25	0
5	0.4	2	1	1.0	1	1.25	3+	7	1.25	0
6	0.4	2	1	1.0	1	1.25	AD	–	–	–
7	0.4	2	1	1.0	1	1.25	AD	–	–	–
8	0.2	2	1	1.0	1	1.25	O	7	1.25	0
9	0.2	2	1	1.0	1	1.25	O	7	1.25	0
10	0.2	1	1	1.0	1	1.25	O			
11	0.2	1	1	1.0	1	1.25	O			
12	0.2	1	1	1.0	1	1.25	2+			
13	0.2	1	1	1.0	1	1.25	AD			
14	0.2	1	1	1.0	1	1.25	AD			
15	0.2	1	1	1.0	1	1.25	AD			
16	0.2	1	1	1.0	1	1.25	AD			
17	0.2	2	1	1.0	1	1.25	O			
18	0.2	2	1	1.0	1	1.25	O			

3+ = Severe dyspnea, convulsions, recovered.
Other abbreviations same as Table I.

TABLE III. NONSPECIFIC HAPTENS (I.P. AND I.V.) DID NOT PREVENT ANAPHYLAXIS FROM SPECIFIC HAPTEN 7 DAYS LATER

GUINEA PIG NUMBER	SENSITIZING ANTISERUM, TYPE II (I.P.) (ML.)	INTERVAL (DAYS)	DESENSITIZATION, NONSPECIFIC CHO, TYPE IV (I.P.) (MG.)	INTERVAL (DAYS)	DESENSITIZATION, NONSPECIFIC CHO, TYPE IV (I.V.) (MG.)	INTERVAL (DAYS)	CHALLENGE, SPECIFIC CHO, TYPE II (I.V.) (MG.)	REACTION
1	0.2	1	1.25*	1	1.25*	7	1.25	2+
2	0.2	1	1.25	1	1.25	7	1.25	2+
3	0.4	1	1.0	1	1.25	7	1.25	AD
4	0.4	1	1.0	1	1.25	7	1.25	3+
5	0.4	1	1.0	1	1.25	7	1.25	1+
6	0.4	1	1.0	1	1.25	7	1.25	AD
7	0.4	1	1.0	1	1.25	7	1.25	3+

*No reaction to I.P. nor to I.V. nonspecific polysaccharide.
1+ = Ruffled hair, slight to moderate dyspnea, huddled, sick-looking, recovered.
Other abbreviations same as Table I.

TABLE IV. SPECIFIC I.P. DESENSITIZATION LASTS AT LEAST 6 DAYS, I.P. DESENSITIZING AND I.V. CHALLENGING DOSES OF HAPTEN OF ONE TYPE DO NOT PREVENT SPECIFIC ANAPHYLAXIS FROM HAPTEN OF THE OTHER TYPE (1 EXCEPTION)

GUINEA PIG NUMBER	SENSITIZING ANTISERUM, TYPE II (I.P.) (ML.)	INTERVAL (DAYS)	DESENSITIZATION, SPECIFIC CHO TYPE I (I.P.) A.M. (MG.)	NOON (MG.)	P.M. (MG.)	INTERVAL (DAYS)	CHALLENGE, SPECIFIC CHO, TYPE I (I.V.) (MG.)	REACTION	INTERVAL (HR.)	CHALLENGE, SPECIFIC CHO, TYPE II (I.V.) (MG.)	REACTION
1	0.25	1	0.5	1.0*	1.0*	6	1.5	0	1	1.5	AD
2	0.25	1	0.5	1.0	1.0	6	1.5	0	1	1.5	AD
3	0.25	1	0.5	1.0	1.0	6	1.5	0	1	1.5	3+
			TYPE II				TYPE II			TYPE I	
4	0.25	1	0.5	1.0	1.0	6	1.5	0	1	1.5	3+
5	0.25	1	0.5	1.0	1.0	6	1.5	0	1	2.0	O
6	0.25	1	0.5	1.0	1.0	6	1.5	0	1	2.0	AD

*Ruffled hair, huddled, sick, recovered slowly.
Other abbreviations same as other tables.

that 2 intra-abdominal injections, 1.0 mg. in the morning and 1.0 mg. in the afternoon, desensitized 12 successive animals. It is interesting that these (Table III) were the only animals in any of these experiments which showed obvious reactions to the intra-abdominal injections of polysaccharide.

Type I polysaccharide desensitized 3 animals completely against a large challenging dose of type I polysaccharide 6 days later. One hour later all 3 animals had typical violent reactions to type II polysaccharide. The reverse was true in the other 3 animals. Namely, type II polysaccharide desensitized completely against challenging doses of type II polysaccharide 6 days later. Two of the 3 animals reacted violently to the challenging dose of type I polysaccharide 1 hour later. No effort was made to explain the failure of the third animal to react.

This same experiment was repeated in its essential details, with one exception. The interval between the desensitizing doses and the challenging specific doses was 9 days instead of 6. In these animals intravenous tests of desensitization were done on the second day. All 12 animals were desensitized. However, 11 days after sensitization and 9 days after the second desensitizing dose, none of the animals reacted to challenging doses of either type of polysaccharide.

Experiment V consisted simply of rechallenging 4 animals (which had been desensitized and had received at least 2 intravenous injections of specific polysaccharide) 25, 30, 30, and 31 days, respectively, after the last dose of polysaccharide. There was no reaction in any of the animals.

DISCUSSION

The data presented show that passive sensitization with a heterologous antibody is transient. This is not new.[4,5] The variable number of days sensitization persists (Table I) is consistent with previous reports.

Pneumococcal polysaccharides, bacterial haptens, can desensitize passively sensitized animals specifically. This is not new.[1] However, rather large divided doses of polysaccharide subcutaneously failed to desensitize 5 animals. Brown[1] reported desensitization with 1.0 mg. subcutaneously in a single dose. One milligram intra-abdominally desensitized less than one-half of the animals in Table II. Eighteen animals in Experiment IV were completely desensitized by 2 or 3 intra-abdominal injections totaling 2 to 2.5 mg. of polysaccharide.

Experiments and Tables II and IV show that desensitization lasts, definitely, from 6 to 7 days. Experiment IV (text) shows that sensitization does not last, reliably, as long as 9 days. From this, the assumption seems reasonable that the desensitization lasts as long as the passive sensitization lasts.

The desensitization is highly specific. Table IV shows this in 5 of 6 animals sensitized with 2 types of antiserum. They had violent reactions when challenged with one specific polysaccharide 6 days after complete desensitization to the other type and one hour after a negative challenge with the desensitizing polysaccharide.

Tomczik[7] showed that the uterine horns of passively sensitized guinea pigs remained sensitive from 2 hours to 12 days. He did not test the intact guinea pig for duration of sensitization. He showed also that polysaccharide could be

used to produce anaphylactic shock. Avery and Tillett[8] showed that polysaccharides will not sensitize anaphylactically, nor produce precipitins. Experiment V confirms this lack of sensitizing capacity.

The impressions are gained that nonspecific polysaccharides tend to lessen the intensity of reactions to specific challenging doses toward the end of the period of sensitization, and that they tend to shorten the duration of sensitization. These impressions need experimental confirmation. No effort was made to confirm them.

It is proper to point out that desensitization with haptens does not imply desensitization to other antigenic components of the pneumococcus. Furthermore, similar experiments may yield different results in animals actively sensitized or sensitized passively with homologous antiserum.

SUMMARY AND CONCLUSIONS

1. In guinea pigs sensitized passively with antipneumococcal rabbit serum, the type specific capsular polysaccharide hapten of the pneumococcus will: (a) elicit anaphylactic shock when given intravenously; (b) desensitize when given intra-abdominally.

2. Passive sensitization with heterologous antiserum is transient. In these experiments sensitization lasted, variably, between 8 and 12 days.

3. Pneumococcal polysaccharides, used as in these experiments, do not produce active sensitization.

4. Desensitization by specific polysaccharide hapten intra-abdominally will: (a) last, apparently, as long as passive sensitization with heterologous antiserum; (b) not prevent anaphylactic reactions to polysaccharide of another type in guinea pigs sensitized to 2 types simultaneously.

5. The roles of active sensitization, passive sensitization with homologous serum, and other haptens and antigenic fractions of the pneumococcus have not been studied.

6. Lasting desensitization of the intact animals is in striking contrast to the transient desensitization of the excised uterine horn reported earlier.[3]

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THE MAJOR ELECTROPHORETIC COMPONENT OF GIANT RAGWEED EXTRACTS: MOLECULAR WEIGHT, CHEMICAL AND BIOLOGIC CHARACTERISTICS*

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ABRAMSON and his associates[1] have described the electrophoretic patterns of whole extracts of ragweed, certain grasses, and tree pollens in a series of publications. In each of these extracts they found a slow-moving, unpigmented fraction, which after separation in the cell was found extremely active when tested in the skin of sensitive patients, and also a rapidly moving pigment of minor specific biologic importance. Between these two, the slowest and the most motile fractions, were boundaries of other pigmented substances, all moving toward the negative portion of the cell. By ultracentrifugation they calculated that the slow or major component separated electrophoretically from ragweed solutions had an approximate molecular weight of 5,000.[2] This fraction gave most of the tests considered characteristics of protein. Sanigar[8] had concluded previously from ultracentrifugal studies of ragweed extracts that they were molecularly heterogeneous and comprised mostly of molecules of weights less than 17,000.

Data indicating that the proteins in ragweed extracts gradually break down into fragments of lesser magnitude have been published previously[3]; at 37° C. the nitrogen in ragweed extracts precipitated by full and half saturation with $Na_2 SO_4$ decreased decidedly within a period of a few days. Stull, Sherman, and Wing[4] have also found that active "protein" fractions precipitated by $(NH_4)_2SO_4$ were retained within a dialyzing membrane. These experiments suggest that molecules larger than 5,000 molecular weight probably exist in these extracts and possibly could be separated from smaller fragments by prolonged dialysis.

The rapidly moving pigment in ragweed extracts described by Abramson has been identified as isoquercitrin.[5] Without drastic methods isolated isoquercitrin could not be freed from biologically active nitrogen, but the nitrogen could be reduced by gentle treatment to a point where reactions in sensitive patients were no longer obtained. The following data are concerned with the material retained within a Visking membrane with a calculated average pore size of 2.5 Angstrom units.

EXPERIMENTAL

Giant ragweed pollen previously washed in ether was extracted in distilled water. The extract was washed, dialyzed, and concentrated in a Visking membrane with buffer solution at pH 8.4 under a pressure of 50 mm. of mercury. Portions of the concentrate within the sac were removed after 10 days of dialysis and dilutions made with buffer mixture were compared as to electrophoretic pattern with the original extract and the dialysate containing 50 mg. N per cent (Fig. 1).

The pollen used in these experiments was supplied by Wyeth, Incorporated.

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A second lot of pollen was treated in the same manner except that M/15 phosphate mixture (pH 7.4) was used throughout. Portions of the concentrate and dialysate were precipitated by full and half saturation with Na_2 SO_4 and with 5 per cent trichloracetic acid, the nitrogen was determined in each precipitate by the micro-Kjeldahl method, and the percentages of precipitable nitrogen in these catagories were calculated for the total material within and for that without the membrane. Sterile dilutions of the original extract, the concentrate, and the dialysate were also prepared for skin testing, and the threshold of the activity of each preparation, based on nitrogen content, was determined.

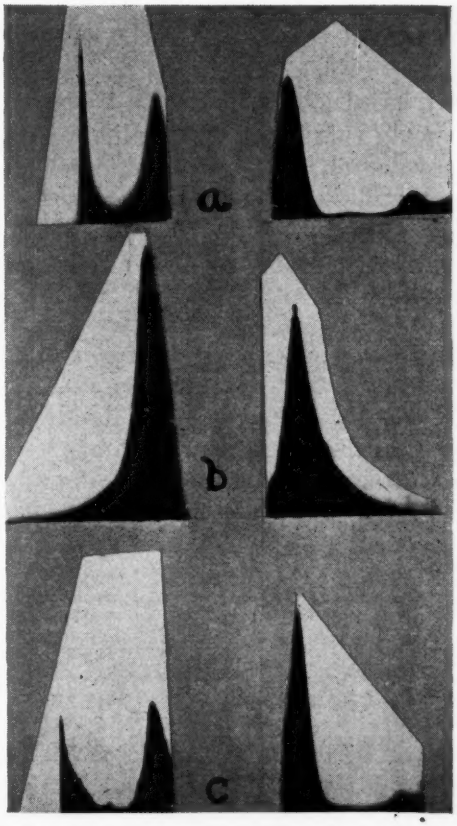

Fig. 1.—Electrophoretic patterns of ragweed extracts; *a*, concentrated dialysate, 50 mg. N/100 c.c., 61 minutes, pH 8.4., mobility of slow component 0.8 cm. $\times$ 10^{-5} volt second; *b*, concentrate within the dialyzing sac, 85.6 mg. N/100 c.c., 180 minutes, pH 8.4, slow component 0.6 cm. $\times$ 10^{-5} volt second; *c*, untreated extract, 50 mg. N/100 c.c., 60 minutes, pH 8.4, slow component 0.6 cm. $\times$ 10^{-5} volt second.

The remainder of the concentrate dialyzed overnight against M/15 phosphate buffer mixture (pH 7.4) was subjected to ultracentrifugal analysis. Sedimentation constants were determined in an air-driven vacuum centrifuge,[6] the sedimentation boundary being recorded by the scanning method of Longsworth.[7] Two analyses were made at room temperature. Since the temperature of the rotor spinning at 48,000 r.p.m. rose about 1.2° each hour, it was possible

to estimate the average temperature over any desired time interval in the run. All values of sedimentation constants were reduced to conditions of pure water at 20°. Diffusion experiments were carried out in a Tiselius cell in M/15 phosphate mixture at 1°. The following data were obtained: D_{20}, 7.1×10^{-7} cm.^{2} per second; S_{20}, 1.3×10^{-13} cm. per second per unit field; assumed partial specific volume, 0.75; frictional ratio, f/f_0, 1.72; molecular weight, M, 17,800, and eccentricity, 1/e, 14. The material appeared to be homogeneous.

DISCUSSION

When the extract of giant ragweed was dialyzed, approximately 85 per cent of the nitrogen passed through the membrane (Table I). The concentrated material within the sausage, light brown in color, was almost one-half precipitated by saturated $Na_2 SO_4$ (Table II), while only 3 per cent of the nitrogen in the dialysate was precipitated by this reagent. It was anticipated that large molecules, if present, would be retained and precipitated in this manner, while those which might be smaller and not precipitable would escape. On the other hand, although very much diluted, slightly more nitrogen in precipitable form was found in the dialyzate than in the concentrate (Table II). Several possible explanations may be given for the escape of this material. The molecular size of the "protein" precipitated by saturation with $Na_2 SO_4$ might be just on the border line of the permeability of the membrane, the pores may have been enlarged by the pressure applied over a period of several days, or as subsequent data suggest, the molecule may be elongated. In some experiments, when extracts were dialyzed at 4° for a period of 10 to 15 days, isoquercitrin crystals actually formed within the substance of the membrane, causing slow leaks. By correlating the ultracentrifugal and diffusion data it was found that the molecules composing the material within the membrane had molecular weights of

TABLE I. PARTITION OF N IN EXTRACTS BY DIALYSIS*

DAYS	INITIAL N (MG.)	CONCENTRATED N (MG. %)	DIALYZED N (MG. %)	RECOVERED N (MG.)
3	25.4	3.6 14	20.5 82	24.1
3	65.2	11.5 19	50.0 76	61.2
7	80.6	7.1 8	71.2 88	78.3
10	99.4	5.9 6	86.0 86	93.5†
10	99.4	6.0 6	87.0 87	94.5†

*In this table, as well as subsequent tables and data, the material within the Visking sausage may be designated as "concentrate" and that without as "dialysate."

†These totals include N in an insoluble precipitate in the concentrated extract, 1.5 and 1.6 mg. respectively, in the last 2 experiments.

about 18,000 and appeared to be elongated with a ratio of length to breadth of 14 to 1.

In a previous publication the rapidly moving pigment described by Abramson was identified as isoquercitrin. This study is concerned only with the slowly moving, unpigmented fraction. A comparison of the patterns of whole extract (Fig. 1), the dialysate, and the concentrated material resulting from long-continued dialysis of the extract shows that a component with the same mobility as that described by Abramson occurs in all 3 and is the only component occurring

in the pattern of the material within the bag. From our observations and those of Abramson it would appear that this component in the pattern of the whole extract is largely a mixture of nitrogen containing molecules of various weights, all with the same mobility. In a mixture of molecules comprised principally of those of low weight not precipitable by saturating with Na_2 SO_4 (Table II), the sedimentation and diffusion boundaries of the sparse molecules of greater weight, such as 18,000, might be obscured and readily overlooked in analyses of whole extracts such as those used by Abramson. From data in a previous communication it was believed that the proteins in the extracts were constantly breaking down into fragments of lesser size, a conclusion that can readily be harmonized with the observations presented here.

TABLE II. N PARTITION OF CONCENTRATE AND DIALYSATE BY PROTEIN PRECIPITANTS

	CONCENTRATE			DIALYSATE		
	TOTAL N (MG.)	PPT. N (MG.)	%	TOTAL N (MG.)	PPT. N (MG.)	%
½ Saturated Na_2 SO_4 (37°)	0.3	0.08	25	4.85	0.08	1.6
5% Trichloracetic acid	0.3	0.08	25	4.85	0.08	1.6
½ Saturated Na_2 SO_4	0.6	0.167	28	9.70	0.2	2.0
1/1 Saturated Na_2 SO_4	0.6	0.27	45	9.70	0.3	3.0

No study of fractions of pollen extracts is complete without correlative observations on the biologic activity of the fractions. Dilutions of the whole extracts, of the material within the dialyzing bags, and of the dialysates of extracts were prepared, using total nitrogen as a standard, so that the thresholds of the activity of the 3 preparations could be compared by testing sensitive patients. Since a tenfold difference between dilutions is usually necessary in cutaneous testing to provoke responses with solutions of different strengths by which the dilutions can be clearly distinguished from each other, the dilutions of these preparations were made in series with each successive dilution 10 times weaker than that preceding. The strongest in each series contained 10 mg. of N in 100 c.c. The material contained within the membrane gave more nearly

TABLE III. CUTANEOUS ACTIVITY OF EXTRACT, CONCENTRATE, AND DIALYSATE

	THRESHOLD REACTION OF EXTRACT (MG. N/100 C.C.)				THRESHOLD REACTION OF CONCENTRATE (MG. N/100 C.C.)				THRESHOLD REACTION OF DIALYSATE (MG. N/100 C.C.)			
PATIENT	10.0	1.0	0.1	0.01	10.0	1.0	0.1	0.01	10.0	1.0	0.1	0.01
E. H.	2+	--			3+	--			--	--		
E. B.			2+	--			2+	--	3+	1+	--	
R. A.			2+	?			3+	?			2+	--
T. C.		1+	--	--			2+	--		1+	--	
R. H.			3+	?			4+	1+	2+	--	--	
P. W. C.			2+	--			3+	2+			3+	--

equal reactions in all patients than did the whole extracts or the dialysates, and patients reacted more strongly to a weaker dilution of this preparation than of the other two. The dialysate was the poorest cutaneous antigen, requiring a stronger dilution to obtain a minimal reaction than was needed with the concentrate or the whole extract. Some patients who reacted to the concentrate and

the whole extract showed no reaction to the strongest solution of dialysate. Although some of the higher molecular material found predominately concentrated in the membrane appeared also in the dialysate, apparently this more active material was diluted with less antigenic nitrogenous fractions until it was no longer potent. The reactions of several of the patients tested have been charted in Table III.

Patients with hay fever have been successfully immunized with the concentrates. From preliminary observations, per unit of total nitrogen, injections of the concentrates of ragweed pollen extracts appear superior to injections of the whole extracts for the prevention of hay fever. The dialysates may give reasonably satisfactory skin tests but still be inferior immunizing agents. Methods useful in obtaining quantities of stable material similar to the concentrate sufficient to immunize a large number of patients have only recently been devised.

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A METHOD OF SENSITIZING GUINEA PIGS TO HORSE SERUM THAT EVENTUATES IN 100 PER CENT MORTALITY AFTER CHALLENGING*

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ASSAYING the value of antihistaminics in protecting guinea pigs from anaphylactic shock would require fewer animals if a method of sensitizing were at hand that invariably led to such a titer of antibodies that the later injection of a challenging dose of the antigen, given after a suitable interval, would always be fatal. The usual methods of sensitizing guinea pigs to horse serum by the intravenous, intramuscular, or intraperitoneal injection of variable amounts of the antigen seldom lead to a fatal degree of sensitivity in 100 per cent of the guinea pigs. Table I, gathered from the literature, is a partial summary of various experiments, giving the number of control animals that died after the challenging dose of the antigen. Only in about one-half of the experiments did the controls prove 100 per cent fatally sensitive, despite the wide variations in the procedural details reported. It is perhaps significant that the 100 per cent fatal sensitivity was usually reported for the smaller control groups. This failure is easily compensated for by the use of more animals, but that would seem unnecessary if a method of sensitization could be devised that would render the guinea pig so sensitive that the ultimate injection of a challenging dose of the antigen would always be fatal. For this reason the present studies were initiated. This problem could, of course, be met by the use of passive sensitization, but for most laboratories this method would probably be more time consuming and less economical.

METHODS

Male guinea pigs weighing from 300 to 350 grams at the time of sensitization were employed in all the experiments. Since the injections of horse serum alone in varying doses, and by varying routes, did not permit the achievement of our purpose, we were led to try 3 other methods: (1) a procedure described by Freund and McDermott,[9] which was originally recommended to us by Richards,[20] and which was an outgrowth of earlier work (see their bibliography); (2) the intrahepatic injection of the antigen[1]; and (3) combinations of the first 2 methods. The method of intrahepatic sensitization was as follows: While an assistant firmly held the animal supine, a 1 inch, 26 gauge needle, attached to a ½ ml. springe, was perpendicularly placed on the skin just to the right of the xyphoid process, and just below the lowest costosternal juncture. The upper end of the syringe was then tilted a

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TABLE I. MORTALITY RATE OF CONTROL GROUPS OF ACTIVITY SENSITIZED GUINEA PIGS IN ANAPHYLACTIC EXPERIMENTS; A PARTIAL SUMMARY OF THE LITERATURE

NUMBER ANIMALS CONTROL GROUP	MORTALITY	PER CENT DEAD	WEIGHT RANGE (GRAMS)	ANTIGEN	DOSE AND ROUTE	SENSITIZING PERIOD* (DAYS)	SHOCKING DOSE	REFERENCES
--	--	90	--	Horse serum	--	--	--	Bernstein and Feinberg[2]
30	24	80	--	Horse serum	0.1 c.c. S. C.	14	0.2 c.c. I. V.	Ercoli and associates[7]
"Large"	--	61	--	--	--	--	--	Feinberg and Bernstein[8]
14	14	100	400-500	Horse serum	0.1 ml. S. C.	12	0.5 c.c. I. V. penile vein	Friedlaender and Friedlaender[10]
--	--	100	--	Sheep serum	2 × 0.5 c.c. after 48 hr. I. P.	15-21	0.1 c.c. I. V.	Halpern[11]
10	10	100	--	Sheep serum	2 × 0.5 c.c. after 48 hr. I. P.	15-21	0.1 c.c. I. V.	Halpern[12]
3	3	100	--	Beef serum	0.5 c.c. S. C.	15	0.1 c.c. I. V.	Halpern[13]
21 (3 groups: 5, 8, 8)	17	81	--	Sheep serum	0.5 c.c. I. P.	23-26	0.25 c.c. I. C.	Landau and Gay[14]
43	37	86	--	Sheep serum	0.5 c.c. I. P.	21	0.25 c.c. I. C.	Landau and associates[15]
12	12	100	--	Egg white	2.0 c.c. of 10 per cent I. P.	21	0.5 c.c. I. C.	Landau and associates[15]
8	8	100	--	Horse serum	--	21	--	Mayer and associates[16]
4	4	100	250-300	Horse serum	2 × 0.5 c.c. I. P. 48 hr. apart	21	0.5 c.c. I. C.	Mayer[17]
13	13	100	--	Horse serum	2 × 0.5 c.c. I. P. 48 hr.	21	1 ml. I. C.	Mayer[18]
13	27	87	300-400	10 per cent horse serum	1.0 c.c. I. P.	about 14	0.5 c.c. I. V.	Reinhard and Scudi[19]
52	43 (table)	83	300-400	10 per cent horse serum	1.0 c.c. I. P.	about 14	0.5 c.c. I. V. jugular vein	Scudi and associates[21]
10	9	90	--	Egg albumin	--	--	I. V. jugular vein	Selle[22]

*The interval between the administration of the sensitizing dose and the challenging dose.
Lacunae (--) indicate the absence of data in the original references.
I.P. = Intraperitoneally.
I.V. = Intravenously.
I.C. = Intracardially.
S.C. = Subcutaneously.

short distance toward the guinea pig's left hind leg so the needle was pointed somewhat laterally and slightly cephalad. The point was then quickly thrust through the skin and muscle into the liver. Its presence therein was indicated by failure to withdraw blood or fluid upon pulling back on the plunger, a peculiar feeling of resistance as the upper end of the syringe was slightly and gently oscillated, and a moderate resistance to the injection. These sensations, as well as the direction in which to insert the needle, are best learned by trial injections into the livers of dead animals with open abdomens. It might be added that, at best, one can never be absolutely sure the needle is in the liver. An occasional animal has been lost during the first day or two after sensitization as a result of hemorrhage or infection. Attempts at injections through the right, lower, and lateral aspect of the thoracic cage led too often to pulmonary complications or intrathoracic injections. The dose injected was 0.05 ml. and if 2 doses were given, the needle was withdrawn from the liver and muscle, and reinserted for the second 0.05 ml. Obviously, sudden and excessive changes in the direction of the axis of the syringe while the needle is in the liver can cause serious or fatal hemorrhage.

The adjuvant employed in this study was killed tubercle bacilli (human 37RV strain made available by our associate, Dr. Philip Eisman), and the vehicles were Aquaphor and paraffin oil, as recommended by Freund and McDermott.[9] Table II gives the proportion of the various materials present when horse serum alone was not employed. It will be noted that the original formula of Freund and McDermott was modified for the intrahepatic injections.

TABLE II. FORMULAE OF ANTIGENIC MATERIAL

TOTAL DOSE (ML.)	EXPERIMENT*	HORSE SERUM (ML.)	AQUAFOR (ML.)	PARAFFIN OIL (ML.)	T. B. ORGANISM (MG.)
0.50	Section B, group II	0.125	0.125	0.25	0.5
0.50	Section B, group III	0.125	0.125	0.25	None
0.05	Sections A & C, group II	0.0165	0.0165	0.0165	0.064
0.05	Sections A & C, group III	0.0165	0.0165	0.0165	None

*Indicates the group in Table III receiving the formula noted.

RESULTS

Table III summarizes the results. The entries are in chronological order. It is difficult to explain why the first 5 groups injected with horse serum alone, given intrahepatically in 2 doses, proved 100 per cent fatally sensitive, whereas survivors were encountered in all subsequent groups but one. Survivors were also encountered in the groups sensitized intramuscularly by the original method of Freund and McDermott. The results would indicate, however, that their formula, with or without tubercular organisms, would yield 100 per cent fatal sensitivity if given intrahepatically or intraperitoneally in 2 doses of 0.05 ml. each.

TABLE III. SECTION A. MALE GUINEA PIGS SENSITIZED BY INTRAHEPATIC INJECTIONS

NUMBER OF GUINEA PIGS	SENSITIZING DOSE (ML.)	INTERVAL IN DAYS*	CHALLENGING DOSE (ML.)	ROUTE OF INJECTION	MORTALITY	PER CENT MORTALITY
		Group I: Horse Serum Alone				
11	0.05	30	0.50	I. C.	7/11	64
9	0.05	21	0.50	I. C.	8/9	89
8	2 × (0.05)	30	0.50	I. C.	8/8	100
6	2 × (0.05)	21	0.50	I. C.	6/6	100
9	2 × (0.05)	22	0.50	I. V.	9/9	100
12	2 × (0.05)	21	0.50	I. V.	12/12	100
11	2 × (0.05)	21	0.50	I. V.	11/11	100
10	2 × (0.05)	21	0.50	I. V.	8/10	80
9	2 × (0.05)	21	0.50	I. V.	6/9	66
11	2 × (0.05)	21	0.50	I. V.	11/11	100
15	2 × (0.05)	21	0.50	I. V.	12/15	80
10	2 × (0.05)	21	0.50	I. V.	7/10	70
9	2 × (0.05)	21	0.50	I. V.	6/9	67
14	2 × (0.05)	21	0.50	I. V.	13/14	93
		Group II: Horse Serum + T. B.				
15	2 × (0 05)	20	0.50	I. V.	15/15	100
17	2 × (0.05)	20	0.50	I. V.	17/17	100
19	2 × (0.05)	21	0.50	I. V.	19/19	100
		Group III: Horse Serum + Vehicles but no T. B.				
24	2 × (0.05)	21	0.50	I. V.	24/24	100
	SECTION B. MALE GUINEA PIGS SENSITIZED BY INTRAMUSCULAR INJECTIONS					
		Group II: Horse Serum + T. B.				
8	0.50	21	0.50	I. C.	8/8	100
11	0.50	21	0.50	I. V.	8/11	73
11	0.50	21	0.50	I. V.	11/11	100
12	0.50	21	0.50	I. V.	10/12	83
26	2 × (0.05)	21	0.50	I. V.	22/26	84.6
		Group III: Horse Serum + Vehicles but no T. B.				
19	0.50	21	0.50	I. C.	18/19	95
19	0.50	22	0.50	I. C.	11/19	58
18	0.50	16	0.50	I. V.	8/18	44
15	0.50	21	0.50	I. V.	15/15	100
13	0.50	21	0.50	I. V.	10/13	77
13	0.50	21	0.50	I. V.	13/13	100
	SECTION C. MALE GUINEA PIGS SENSITIZED BY INTRAPERITONEAL INJECTIONS					
		Group II: Horse Serum + T. B.				
18	2 × (0.05)	21	0.50	I. V.	18/18	100
		Group III: Horse Serum + Vehicles but no T. B.				
19	2 × (0.05)	21	0.50	I. V.	19/19	100

*Refers to days elapsing after sensitizing before challenging.
I.C. = Intracardiac
I.V. = Intravenous
T.B. = Killed tubercle baccilla

Table IV summarizes the protective action exerted by 2 antihistaminics, 2-phenylbenzylaminomethyl imidazoline hydrochloride (Antistine), and N,N-dimethyl-N′-benzyl-N′-α-pyridyl) ethylenediamine monohydrochloride (Pyribenzamine), against fatal anaphylactic shock in guinea pigs sensitized by the modification of Freund and McDermott's method.

Even very high doses of the antihistaminics protected few animals but the animals that died usually survived much longer than the controls.[6]

DISCUSSION

Richards originally recommended the method of Freund and McDermott because up to that time it had never failed, in his laboratory, to yield a potentially fatal level of antibody. In our hands this was not always the case, and more recently Richards[20] has also noted exceptions (Table III, Section B, Group II). The method of intrahepatic injections was initially employed on the assumption that a direct delivery of the antigen to such a large concentration of reticulo-endothelial elements as possessed by the liver might lead to a higher concentration of antibodies, especially in the blood itself. The injection of only a single dose intrahepatically (Table III, Section A, Group I, first 2 experiments) failed to yield the required level of antibody. Presumably a minimal dose is required, as has been shown by Coulson and Stevens[3-5] (as well as others), but once attained, further increases in the volume of a single injection do not materially increase the production of antibody. The injection intrahepatically or intraperitoneally of the formula of Freund and McDermott (with minor modifications) led to a 100 per cent potentially fatal level of antibody (Table III, Section A, Group II; Section C, Group II). The success of the method would not appear to validate the assumption that led to its trial. The injection intrahepatically or intraperitoneally of the same formula without the organisms also caused a potentially fatal degree of sensitivity (Table III, Section A, Group III; Section C, Group III). If, in additional experiments, these same results were obtained, it would indicate the intrahepatic and intraperitoneal administrations of the antigen were superior to the intramuscular and intravenous in respect to the titer of antibodies produced in the blood. This might be due to the more rapid access of the injected antigen to the liver via the portal circulation, but this speculation might be difficult to establish experimentally.

TABLE IV. EFFECT OF ANTISTINE HCl AND PYRIBENZAMINE HCl ON ANAPHYLACTIC SHOCK IN MALE GUINEA PIGS (ADMINISTERED I. P. 30 MINUTES BEFORE ANTIGEN)

	ANTISTINE HCl						
NUMBER OF PIGS	DOSE MG./KG.	CONCENTRATION PER CENT	ROUTE	HORSE SERUM (DOSE C.C.)	ROUTE	NUMBER OF DEATHS	PER CENT MORTALITY
10	28	2	I. P.	0.50	I. V.	10/10	100
8	42	2	I. P.	0.50	I. V.	5/8	62.5
	PYRIBENZAMINE HCl						
10	14	2	I. P.	0.50	I. V.	10/10	100
9	21	2	I. P.	0.50	I. V.	6/9	66.6
	CONTROL GROUP						
6	--	--	--	0.50	I. V.	6/6	100

The antihistaminics protected from death only about one-third of the animals sensitized by the intrahepatic injection of a modified Freund and McDermott formula (Table IV). This might be due to one or both of the following factors: (1) substances other than histamine are released which threaten the life of the animal in anaphylactic shock, (2) such tremendous quantities of histamine are released by the injection of the challenging dose that cardio-

vascular collapse results. It has, of course, been repeatedly demonstrated that antihistaminics will only partially antagonize the hypotensive effects of large doses of histamine.

The value of the method described for assaying the activity of antihistaminics in anaphylactic shock is debatable. The method assures a potentially fatal titer of antibody but it does not permit adjudging, without precipitin tests, how many multiples of a minimally fatal titer may be present. The animals dying despite the antihistaminic did survive much longer than the controls (from 2 to over 70 times as long) and the use of the survival time might allow a more accurate comparison of the activities of 2 potent antihistaminics. The method might prove useful in detecting the action of potential therapeutic agents that interfere with, or prevent, the antigen-antibody reaction, but at present we know of no such drugs.

SUMMARY

Various methods of sensitizing guinea pigs to horse serum were employed in the hope of finding one that would invariably produce a potentially fatal level of antibody in 100 per cent of the animals. The only successful method involved intrahepatic or intraperitoneal injections of 2 × 0.05 ml. of horse serum alone, or with killed tubercle organisms. The details of this new method have been described. Even large doses of antihistaminics offered but partial protection against anaphylactic shock to animals so sensitized. This might be additional evidence that released histamine does not alone account for the symptoms of anaphylactic shock, but the possibility was not excluded that so much histamine was liberated that cardiovascular collapse resulted, since it is known that antihistamines only partially antagonize the hypotensive actions of higher doses of histamine.

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GRANULOMA OF THE SPACE OF RETZIUS WITH EOSINOPHILIC INFILTRATION IN AN ALLERGIC PATIENT*

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EOSINOPHILIC GRANULOMA is a term usually applied to solitary or multiple circumscribed granulomatous lesions of bone in which there is marked infiltration with eosinophils, as well as to similar but less sharply circumscribed lesions of other organs. As a distinct clinical entity, the solitary eosinophilic granuloma of bone was described first in 1940 by Otani and Ehrlich,[1] and by Lichtenstein and Jaffe.[2]

In 1946, Dundon, Williams, and Laipply[3] published a summary of the literature on eosinophilic granuloma with a presentation of 53 cases, pointing out that most authors considered the lesion to be of inflammatory type, although the causative organism had not been demonstrated. In their series of cases the majority of lesions were confined to bone, although extraosseous eosinophilic granulomas were noted as well. The granulomas of bone were usually solitary, involving the skull, pelvis, vertebrae, ribs, and long bones. Multiple lesions of bone were present in 10 of the 53 recorded cases. The extraosseous granulomas mentioned involved the mediastinum and lymph nodes. Systemic manifestations present with the solitary or multiple eosinophilic granulomas were sometimes slight fever, weight loss, anorexia, and easy fatigability. There sometimes was an increased sedimentation rate, as well as a leucocytosis and eosinophilia.

In Dundon's description of eosinophilic granulomas, the microscopic appearance varied so that it was felt that 3 stages existed in the disease. In the early stage, the granuloma was cystic with necrosis, hemorrhagic areas, and an infiltration of many eosinophils. In the intermediary stage there were few eosinophils, but many mononuclear histiocytes (foam cells). In the late stage there was proliferation of connective tissue and regeneration of bone. The presence of foam cells, as well as other factors, suggested a connection between eosinophilic granuloma, Letterer-Siwe, and Hand-Schüller-Christian diseases.

Because of the similarities in these 3 disorders, Parkinson,[4] in 1949, stated that it was accepted that they were phases of the same disease. This conclusion was based on the fact that local granulomas of bone may be present in a generalized disorder affecting the skeleton and viscera. Conversely, in the generalized reticuloses (Hand-Schüller-Christian and Letterer-Siwe diseases) there may appear bony changes radiographically and histologically identical with those of eosinophilic granuloma. Mallory,[5] in 1942, had concluded that the disease occurred in infancy as Letterer-Siwe disease, in childhood as Hand-Schüller-Christian disease, and in older children and adults as eosinophilic granuloma. Jaffe and Lichtenstein,[6] in 1944, had felt that the histopathology present in the individual case depended upon the duration of the disease.

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Parkinson, therefore, with his case which had evidence of pulmonary, pituitary, and bony disease, recommended that the name eosinophilic xanthomatous granuloma should be adopted for the generalized form of the 3 conditions.

Paralleling the later development of this concept of eosinophilic granuloma there began to appear indications that this lesion might be more widespread in its occurrence than had been indicated, and that there might be other factors involved in its pathogenesis.

Eosinophilic granuloma of the skin was described in 3 cases by Lever, Lane, Dowing, and Spangler,[7] who pointed out that the relationship of such lesions of the skin to eosinophilic granuloma of bone was not well defined.

An unexplained bilateral pulmonary infiltration was noted by Dickson[8] in a case of eosinophilic granuloma of bone. There are other recorded cases of pulmonary infiltration in "eosinophilic xanthomatous granulomas."[9-11]

A case in which eosinophilic granuloma occurred in the jaw as well as in the gums and palate, with a simultaneous fistula in ano, was described by Schroff.[12]

There have been reported cases of eosinophilic granuloma of the intestine in which the etiology was not apparent. Vanvek[1] described cases of gastric submucosal granuloma with eosinophilic infiltration. The lesions seen were thought not to be identical with eosinophilic granuloma previously described in bone and soft tissue. It was pointed out that the basic cell of the granuloma of eosinophilic granuloma of bone and soft tissue was thought to be the histiocyte or reticulum cell, while the basic cell of the gastric submucosal granuloma was probably the fibroblast. In the cases of Vanvek there was uncertainty as to whether or not the granulomas were due to an inflammatory process. Since the only outstanding feature was the eosinophilic infiltration, the question of an allergic process was raised, but no definite evidence of allergic factors was found upon clinical review of the cases.

Kaijser[14] described a resection of the stomach for ulcer symptoms whereupon thickening of the pylorus by an inflammatory process was found with an eosinophilic infiltration. This patient gave a family history of allergy. In his youth onion was said to have caused vomiting. There was a 25 per cent eosinophilia found in the differential count of the leucocytes. Two other cases of eosinophilic granuloma of the intestinal tract were described in which allergic factors were not noted. In 1947 Ackerman[16] described a case of eosinophilic granuloma with involvement of the lungs and diaphragm. In his case, a biopsy of an involved rib showed marrow with the fat cells crowded out by eosinophils and a few macrophages. Ackerman pointed out the lack of understanding of the relation of the histologic pathology of visceral and bone eosinophilic granulomas. He noted that in the literature there was a discrepancy between the histopathologic appearance of the osseous and visceral lesions and that it had not been considered of paramount importance. He stated that there were no reports of visceral lesions dominated by eosinophils equivalent to those seen in the bones, but that most writers agreed that the basic disorder in the Hand-Schüller-Christian type diseases and bone eosinophilic granuloma was a "peculiar

inflammatory histiocytosis.'' Unfortunately, in Ackerman's case tissue from the diaphragmatic tumor was not obtained for study. Ackerman concluded that the stages described in the histopathologic appearance of the lesions presented in the literature might be variations in the evolution of the basic pathologic process.

The following case is presented as one in which an unusual eosinophilic granuloma occurred in a patient with a long-standing history of allergy. In addition, there was suspicion of a diffuse vascular disease present of the type of allergic disorder of the blood vessels described by Harkavy[17] in 1941. Also, there were observed acute infiltrative processes of the lungs which have been described so frequently in the literature in connection with the problem of eosinophilic granuloma. This 60-year-old Italian housewife presented a problem in regard to the diagnosis and treatment of eosinophilic granuloma because of the site of the tumor in the space of Retzius, its large size causing symptoms of pressure upon the neighboring organs, and because of coexisting severe bronchial asthma.

Born in Italy, this patient, L. P., came to the United States in 1906 when she was 16 years of age; she has lived continuously in the Staten Island area. She could not recall childhood illnesses or previous allergic diseases. Her first pregnancy in 1911 was complicated by severe edema. It terminated spontaneously after 5 months, the delivery being followed immediately by convulsions. There was a subsequent pregnancy under close medical supervision which resulted in a full-term normal delivery without complications.

She was in good health except for frequent colds until the first sign of her approaching infirmity occurred in 1944 at the age of 54 years. At the time she noted ''tightness of the chest'' during an attack of acute bronchitis. There was slight asthma during 1945. In April of 1946, following a winter of frequent upper respiratory tract infections, more severe asthma began. Acute tonsillitis occurred in June of that year. One month later a tonsillectomy was performed. Hospitalization was necessary again in September and in November because of asthma and acute upper respiratory tract infections. A few skin scratch tests at that time were said to be positive for certain foods. The asthma, which was continuous but varying in intensity, was not relieved by an elimination diet. She was in violent status asthmaticus following intratracheal lavage with a solution of penicillin. She had been under treatment with injections of autogenous vaccine in dosages as large as 10 c.c.; the concentration of this seemingly massive dose is not known. When transferred to Roosevelt Hospital in January of 1947, severe asthma was present upon admission. A bilateral maxillary sinusitis was present. There was a resolving bronchopneumonia of the right lung. After supportive and penicillin therapy, her condition improved.

A bilateral Caldwell-Luc operation was performed 4 weeks after admission. At operation the membrane in the right antrum showed moderate polypoid change and there was also polypoid change in the membrane of the left antrum. Several of the small polyps showed cystic degeneration and abscess formation. In the cavity of the left antrum there was found a large clump of tenacious gelatinous mucopus. The membrane was moderately vascular in both antra. Microscopic examination of the sinus membrane showed edema and an eosinophil infiltration, as well as many round cells. The mucus gland cells were increased and active. There was a definite hyaline membrane under the epithelial cells. Culture of the antral tissue grew pneumococci. Culture of an abscess in the left antrum grew hemolytic staphylococci and an indifferent streptococcus.

Intradermal tests with dust concentrate gave slight reaction. Intradermal tests to the following substances were negative; timothy, rabbit epithelium, feathers, orris, kapok seed, dog epithelium, ragweed, horse epithelium, karaya gum, wool, cottonseed, cat epithelium, plantain, horse serum, pyrethrum, flaxseed, and a mixture of 7 mold extracts.

There was a marked blood eosinophilia during this hospital admission. In a total leucocyte count of 14,400 there were 65 per cent polymophonuclear leucocytes, 19 per cent eosinophils, 14 per cent lymphocytes, and 2 per cent monocytes. In another count of 16,600 leucocytes there were 22 per cent polymorphonuclear leuccocytes, 55 per cent eosinophils, 20

per cent lymphocytes, and 3 per cent monocytes. A muscle biopsy showed changes in the muscle fibers said to be suggestive of dermatomyositis. There were also vascular changes suggestive of periarteritis nodosa, but the evidence of systemic disease was uncertain since the patient was thought to have received injections of adrenalin in oil at or near the site of the biopsy. While the patient was in the hospital an attempt was made to institute herapy with autogenous vaccine. Dilute quantities of the vaccine seemed to produce asthma so that the injections were discontinued.

At the time of discharge asthma was quiescent and remained so while the patient visited Arizona and New Mexico, but nasal occlusion was troublesome during this 4-month period. A bilateral ethmoidectomy was done at the Roosevelt Hospital in November, 1947.

Early in 1949 asthma recurred with increasing severity. At the time of the patient's third admission to the Roosevelt Hospital in June, 1949, at the age of 59 years, therapy consisted of deep anesthesia with ether for 30 minutes, followed by bronchoscopy. Congestion of the entire bronchial tree was noted. Moderate amounts of thick mucopurulent material were aspirated.

In October, 1949, the patient sought the advice of another physician. During the ensuing 6 months, over a dozen bottles of a medicine said to contain iodide and arsenic among other ingredients were consumed. There was temporary relief of the asthma at the start of this therapy. In January, 1950, another acute upper respiratory tract infection with asthma made necessary 12 days of bed rest. Aureomycin was taken at this time.

In February asthma was still controlled with difficulty. In addition there was dysuria, frequency, small urinary stream, and pain about the urethra. The urine gradually became scanty until anuria was present. Cystoscopy was performed at St. Vincent's Hospital.

An indwelling catheter was left in place for 3 days. Upon its removal, the patient could void again. However, in a few days time catheterization of the bladder was again necessary.

At the time of admission to the Roosevelt Hospital for the fourth time, acute urinary retention was present. Temperature was 101.8° F., pulse 110, respirations 35, and blood pressure 112/70. There was status asthmaticus. The patient was coughing frequently with the production of small amounts of mucopurulent sputum from which were cultured *Streptococcus viridans* and *Neisseria catarrhalis*. There was reddening of the pharynx and the lungs were emphysematous. Loud sibilant and sonorous sounds were heard in both lungs. The heart was enlarged to the left. Rhythm and rate were regular and no murmurs were heard. Tenderness to deep palpation of the lower abdomen was present. A firm, nontender, rounded mass rising to the umbilicus was palpable in the abdomen. There was slight pitting edema of the ankles. Pelvic examination revealed an edematous urethra with a small caruncle at the orifice. After catheterization of the bladder the abdominal mass partly disappeared, but the bladder was judged to be displaced upward. The uterus was not enlarged. A symmetrical irregularity was palpable anterior and to either side of the bladder. This mass was of spongy consistency and extended the entire length of the urethra.

Laboratory findings were as follows: the initial urine specimen contained 2 plus albumin, 2 plus acetone, with an occasional red blood cell and white blood cell. Total leucocyte count was 13,275 with a differential of 66 per cent polymorphonuclear leucocytes, 12 per cent lymphocytes, 21 per cent eosinophils, and 1 per cent monocytes. The sedimentation rate was 23 mm. (Wintrobe). Cell volume was 38 per cent. Hemoglobin was 12.3 Gm. Platelets were adequate. Blood urea nitrogen was 5.5 mg. per 100 c.c. and fasting blood glucose was 92 mg. per 100 c.c. Culture of the urine grew coliform organisms resistant to penicillin and dihydrostreptomycin with one plus sensitivity to aureomycin and 4 to Chloromycetin. The cephalin flocculation reaction was 3. An electrocardiogram showed left axis deviation.

A biopsy of the left calf muscle was done. Examination of the tissue* revealed striated muscle. Specific inflammatory cell infiltration was absent. Sections of an artery showed moderate intimal sclerosis. In one vein there was obliteration of the lumen with necrosis in the wall. There was rich infiltration with lymphocytes and a few neutrophils and eosinophils in the surrounding stroma.

*By Dr. W. W. Brandes.

Roentgenograms of the spine and long bones gave negative findings. An x-ray examination of the chest showed cardiac enlargement, mainly of the left ventricle. There was diffuse exaggeration of the bronchovascular markings, particularly at the right lung base. An intravenous pyelogram and cystogram showed the bladder to be of normal size, but the entire organ was elevated in the pelvis. The kidney pelvi were not visualized.

Two days following admission cystoscopy and urethroscopy were performed. Helium-gas-oxygen-ether anesthesia was used. After a stormy induction, the pelvis was explored and a suprapubic cystostomy and anterior colpotomy were performed. Papilledema of the entire

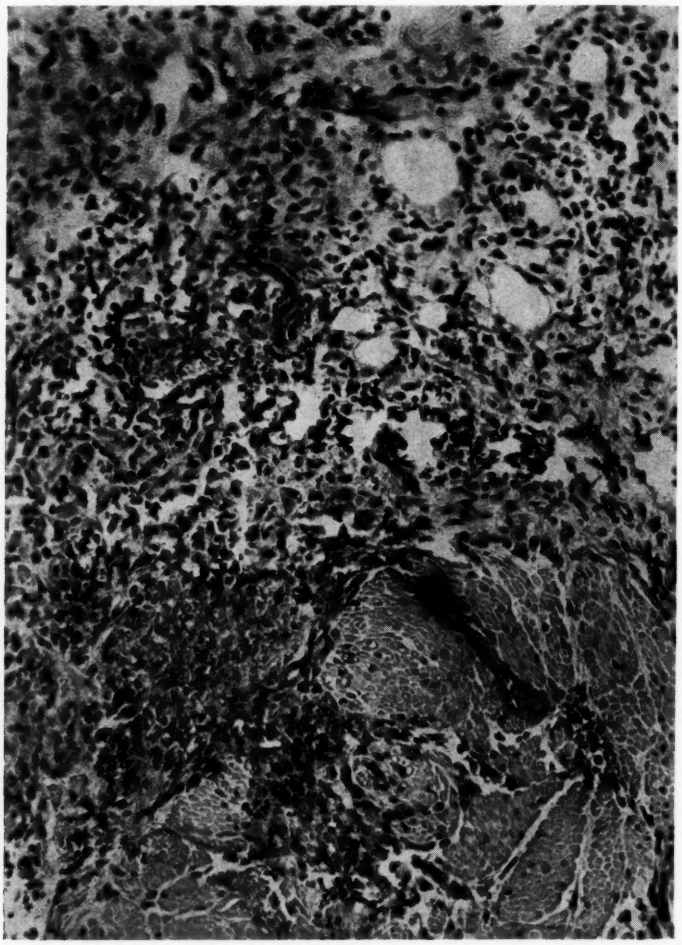

Fig. 1.—Section of granuloma tissue removed at biopsy.

bladder floor was seen. A firm, indurated mass encircled the urethra. The mass was the size of a medium-sized orange and encroached upon the lower aspect of the bladder wall. The immediate report of a frozen section of the mass was inflammatory tissue.

Through an incision in the anterior vaginal wall, a large bore needle was inserted into the mass, but upon several attempts the operator was unable to recover pus. Several biopsy specimens were taken from the main portion of the mass. Biopsy showed dense fibrous tissue with muscle fibers present, separated by bands of inflammatory tissue in which were areas of hyalin necrosis. Fibroblasts, macrophages, lymphocytes, plasma cells, and numerous

eosinophils were present. The walls of some blood vessels were thickened with hyalin degeneration and infiltration with inflammatory cells. The diagnosis was allergic, or eosinophilic, granuloma.

Following surgery, asthma was improved for a few days. Two transfusions of whole blood were required because anemia was present. The blood eosinophilia persisted. It was judged that the mass was slightly diminished in size. An attempt to remove the suprapubic catheter and introduce a French 22 Foley catheter by way of the urethra was not successful.

X-ray therapy was administered over a period of 5 days, 300r daily, to the area of the tumor. Four days later micturition was performed normally through the urethra with only

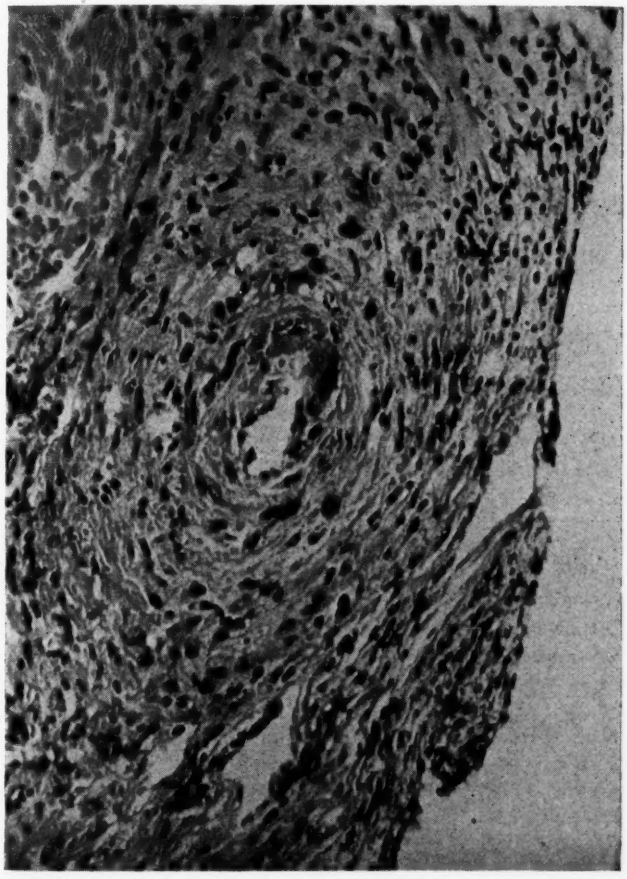

Fig. 2.—Small artery incorporated in granuloma tissue.

slight terminal burning. Seven days later the mass had diminished greatly in size. By the end of another week the mass had disappeared, as well as that part of it which surrounded the urethra. There was no growth of bacteria in the urine following procaine penicillin, Chloromycetin, and terramycin therapy.

Since her admission to the hospital, the patient had continuous asthma. This was partially relieved only during the 4 days immediately following the operation, after which it recurred with the same severity as before so that frequent injections of aminophylline and Adrenalin were necessary. The lung fields showed a diffuse, chronic, interstitial peribronchial alteration with slight confluence at the bases which tended to increase upon one occasion when a temperature elevation occurred, but otherwise constantly tended toward clearance. The

cardiac silhouette decreased but remained at the upper limit of normal in size. Cortisone, 100 mg. daily, was started. Under this therapy the asthma abated in 5 days. A maintenance dose of cortisone, 25 mg., was continued for 40 days. At the end of this time the total leucocyte count was 7,250 with a differential count of 58 per cent polymorphonuclear leucocytes, 30 per cent lymphocytes, 10 per cent eosinophils, and 2 per cent monocytes. While the patient was under cortisone control, autogenous vaccine was given in appropriate dosage without the occurrence of asthma. Following the discontinuance of cortisone the patient's sensitivity to vaccine returned and this therapy had to be stopped.

The patient was asthma free and voiding normally at the time of discharge from the Roosevelt Hospital, 3 months after her admission. At this time blood eosinophilia was 11 per cent, or a total of 681 cells. The vital capacity had increased from 900 c.c. to 1,200 c.c.

One week after discharge asthma began to recur. She was readmitted to the Roosevelt Hospital July 28, 1950, for re-evaluation of the asthma and further treatment. In addition to asthma, there were complaints of frequency of urination and burning. Cystoscopy performed a few days after admission revealed only moderate inflammatory changes of the bladder mucosa.

Examination of a urine specimen at the time of admission showed no abnormality except for a few pus cells. Fasting blood glucose was 127 mg. per 100 c.c. Blood urea nitrogen was 5.0 mg. per 100 c.c. The blood Kline and Mazzini tests were negative. Hemoglobin was 13.4 Gm. Total leucocyte count was 13,750 with 62 per cent polymorphonuclear leucocytes, 12 per cent eosinophils, and 26 per cent lymphocytes. Sedimentation rate was 22 mm. (Wintrobe). Urine culture again showed coliform organisms which were resistant to penicillin, aureomycin, and terramycin but not to Chloromycetin.

There was no evidence of the tumor found upon pelvic examination. A roentgenogram of the lungs showed the same diffuse, chronic interstitial peribronchial change as during the previous admission. Fever and urinary tract symptoms subsided after Chloromycetin therapy was instituted but asthma persisted. ACTH was started in dosage of 20 mg. every 6 hours and there was marked improvement of the asthma in 3 days. The ACTH was continued in dosage of 10 mg. every 6 hours for 9 days. The vital capacity at the end of this period had increased from an initial 1,000 c.c. to 2,000 c.c. The total blood eosinophil count varied throughout the course of ACTH from 763 to 3,181 cells. When the injections of ACTH were stopped, cortisone was started and the patient was sent home on a maintenance dose of cortisone of 25 mg. daily.

While the patient was at home she did well on this schedule of cortisone injections, although her activity was restricted to the extent that she did a minimum amount of housework. A dentist was consulted for the purpose of doing extractions of numerous teeth which were in poor condition. During this time cortisone injections were discontinued for 8 days and the extractions were not completed, apparently due to the feeling that there would be improper healing of the jaw and gums because of the cortisone therapy. On the fifth day after the cortisone was stopped, the patient again began to have asthma. Although cortisone was begun again, the asthma never entirely cleared and the patient had to have injections of Adrenalin daily.

Admitted to the Roosevelt Hospital for the purpose of completing the dental extractions, the patient was found to have moderate asthma. The vital capacity was 1,000 c.c. Urinalysis was negative. The leucocyte count was 6,800 cells, with a total eosinophil count of 319 cells. The sedimentation rate was 16 mm. and the hemoglobin 14.5 Gm. A roentgenogram of the chest showed no change from the last film taken during her previous admission. During her 6-day admission in the hospital, penicillin by injection and cortisone, 50 mg. daily, were given. The extractions were completed and no delay was observed in the rate of healing of the gums. Asthma improved so that injections of Adrenalin were no longer necessary. Upon her discharge, the maintenance dose of 50 mg. of cortisone daily was continued and asthma was adequately controlled by this dosage.

Separation of the components of the blood serum was done by electrophoresis on specimens of blood taken from this patient at varying intervals throughout the course of her illness. The gamma globulin fraction, expressed as the percentage of total serum protein, was found

to be increased above the normal in most instances. The alpha$_1$ and alpha$_2$ fractions constantly were increased above the normal, while the albumin fraction was depressed. These data, with a discussion of their significance, are to be presented elsewhere.

DISCUSSION

Taking into consideration the past efforts at clarification of the etiology and the attempts at systematization of the description of the histopathology of eosinophilic granuloma, it cannot be said that the evidence present in this case offers more in the solving of the problem than the presence of the lesion in a patient who is suffering from the manifestations of chronic bacterial allergy causing mainly severe asthma. The acute infiltrative lesions in the lungs may have been areas of bronchopneumonia or may have been the Löffler type of eosinophilic infiltration. The result of the chronic infection of the nasal passages and sinuses was a hypertrophic sinusitis. The presence of diffuse vascular disease of the type described by Harkavy was suspected following biopsy of muscle on two occasions. The persistent blood eosinophilia was subsantial evidence that a severe generalized allergic disease was present. In all possibility the eosinophilic granuloma was one other manifestation of the generalized disease.

In a case of chronic asthma presented by M. M. Melicow at a recent meeting of the New York Allergy Society, an allergic granuloma similar histologically to the tumor described here was found in the prostate. In Melicow's case eosinophilic granulomatous lesions were found also in the bladder, esophagus, rectum, seminal vesicles, and heart. Eosinophilic infiltration, Löffler type, was present in the lungs at autopsy.

The relation of the eosinophilic granuloma in these cases to the solitary or multiple eosinophilic granulomas of bone originally described in 1940 is not clear. The eosinophilic xanthomatous granulomas such as Hand-Schüller-Christian and Letterer-Siwe diseases have been thought to arise from basic defect of the reticuloendothelial system. There is evidence that antibody production with its clearly allied allergy processes is also related to the reticuloendothelial system. To suggest that these two apparently dissimilar complexes therefore are connected is premature at this time.

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SENSITIVITY TO SESAME SEED

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A THOROUGH review of the literature reveals no published reports of sensitivity to sesame seed prior to a case described recently by Rubenstein.[1] In this case, attacks were brought on by ingestion of a confection known as Halvah, which contains sesame seed and oil, and also by Italian-French bread containing sesame seed. Outstanding in the allergic reaction were the picture of shock and the symptoms of edema of the glottis. Intense erythema and itching of the skin were also prominent.

Because of the scarcity of recorded cases, I believe it would be of interest to report an additional case of sensitivity to sesame seed.

CASE REPORT

H. Z., a 40-year-old white man, was first seen in March, 1950, complaining of infrequent attacks of angioedema of the lips, tongue, and uvula associated with cough and wheezing. Family history for allergy was negative. Past history revealed no allergic disorders prior to 1940. In 1940, the patient had several attacks of urticaria. These attacks were comparatively mild and abated quickly and spontaneously. Their cause was not determined. Between 1942 and 1946, while the patient was serving with the armed forces, there were no attacks of urticaria.

In March, 1950, the patient was seen with marked angioedema of the lips, tongue, and uvula. There was also a hacking cough; sibilant sounds were present in both lungs. He was given 4 minims of Epinephrin 1:1000 subcutaneously with partial relief in 20 minutes. An additional 4 minims quickly cleared both the angioedema and asthmatic symptoms.

On questioning the patient it was learned that about 30 minutes prior to his being seen, he had eaten a piece of Halvah. Within 10 minutes after ingestion, he began to feel a burning and tingling sensation of the lips and throat. By the time he reached the office (½ hour after ingestion), the symptoms of angioedema and asthma had fully developed. At this time the patient volunteered the information that he had had several previous attacks after eating Halvah as well as after partaking of a particular home-baked pastry.

A few days after this episode, studies were begun to determine the etiologic factors involved. It had been ascertained that the brand of Halvah eaten by the patient contained crushed sesame, corn syrup, sugar, vegetable oil, egg albumin, cocoa, and chocolate. The pastry that precipitated the attack contained the following ingredients—flour, baking powder, vegetable oil, eggs, honey, sugar, and sesame seed. Intracutaneous tests of various foods, including all ingredients of both Halvah and the pastry in question, were performed with the following results: there were moderate reactions to chocolate, oats, tomato, codfish, coconut, grapefruit, tea, and pineapple; slight reactions were obtained to wheat and cottonseed; negative reactions were shown by egg albumin, peanut, and corn. Serial testing with sesame seed extract, 1 protein nitrogen unit per cubic centimeter and 10 protein nitrogen units per cubic centimeter produced a slight reaction with the former and a moderate reaction with the latter. Thereupon an intracutaneous test with a sesame seed extract 100 protein nitrogen units per cubic centimeter was performed and showed a marked active reaction.

Passive transfer tests were done and showed identical reactions in every case as were obtained by direct testing.

Clinical trial of all positively-reacting foods was now begun. Although the patient's diet had not been restricted, except for sesame seed, during the period required for skin testing, and there had been no attacks of angioedema or asthma, it was nevertheless decided to eliminate all positively-reacting foods for one week and then to re-introduce these foods into the diet one at a time. Each food was given in more than ordinary amounts on 2

successive days and then withheld for 3 days. If no symptoms occurred, the procedure was repeated. If no symptoms then developed, the food was considered nonallergenic and added permanently to the patient's diet. In addition, any food that gave a negative skin test but was an ingredient of either Halvah or the aforementioned pastry was tested clinically in the same manner. In this group were eggs, corn syrup, honey, and the various vegetable oils including corn oil, cottonseed oil, peanut oil, and olive oil. None of these foods precipitated asthma, angioedema, or urticaria on clinical trial.

With the cooperation of the patient, it was decided to verify the apparent clinical sensitivity to sesame seed. A No. 1 pink capsule had been filled with 0.3 Gm. of the seed. This was swallowed by the patient while in the office, and he was asked to wait until it could be observed whether or not any reaction would develop. Within 25 minutes, there was marked tingling and burning of the lips and throat, and slight puffiness of the lips. The reaction was not allowed to proceed further but was brought under control by an injection of 5 minims of Epinephrin 1:1000 subcutaneously. In order to rule out any psychic element, the test was repeated at weekly intervals using poppy seed and caraway seed as controls. In neither case was there a reaction. When the test was repeated with sesame seed, the angioedema again developed. At no time was the patient informed as to what seed was used; nor could he have known what seed was used inasmuch as all capsules were the same in size and color.

With scrupulous avoidance of any food that might contain sesame seed, the patient has remained entirely symptom-free.

COMMENT

A case of angioedema and asthma due to the ingestion of sesame seed is presented. Judging from the scarcity of reports in the literature, such sensitivity is rather uncommon. However, when it does occur, the symptoms may be alarming.

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Editorial

MATERIALS FOR SKIN TESTING

In the minds of the public and the great majority of the medical profession, an allergist is a doctor who does skin tests. Much as the allergist may seek to broaden his diagnositic approach and exercise clinical judgment, his main distinction from the remainder of his profession is in his ability to discover the specific causes of allergic disease by demonstration of positive skin reactions, and his usefulness is judged to a great degree on the accuracy of his testing methods. An etiologic diagnosis backed by a positive skin test appeals to both patient and family doctor, while a diagnosis made on clinical grounds in the face of a negative test may be considered only a shrewd guess, to be accepted or rejected depending upon whether or not it agrees with one's own opinion.

It therefore behooves the practitioners of allergy to examine critically the methods used in performing skin tests in an effort to obtain the greatest possible correlation between skin reactions and clinical sensitization. In general, skin tests with pollens and inhalants, when properly performed and interpreted, yield sufficiently reliable results to be of great value in diagnosis. On the other hand, skin tests with foods give so many reactions, both "false positives" and "false negatives" which do not agree with the clinical symptoms, that many physicians, both allergists and others, have come to the belief that they are of little or no value.

Some of these inconsistencies are explainable. A positive skin reaction to a food which the patient can eat with impunity may mean that the antigenic properties of the original food have been so changed in cooking, digestion, adsorption, and metabolism that no significant amount of active antigen reaches the shock organ through the circulation. Some delayed clinical allergic reactions to foods associated with negative skin tests have been shown by Cooke[1] to result from sensitization, not to the unaltered food, but to a proteose or other product of its digestion. In such cases, skin tests with suitable preparations of the artificially digested food give positive reactions.

However, evidence is gradually accumulating that failure to demonstrate positive skin reactions in certain clear-cut cases of clinical food allergy is not due to changes in the antigens as a result of digestion and metabolism, but simply to failure to employ the antigens in an active form for skin tests. Many an allergist has been embarrassed by his inability to demonstrate positive skin reactions in patients who know from repeated experience that they develop allergic skin rashes after eating berries or shellfish. Tuft and Blumstein[2] in 1942 showed that the antigens of berries and certain other fruits were very easily destroyed in the preparation of extracts for skin testing. In patients known to be sensitive to these foods, in whom skin tests with the available extracts gave no reaction, the allergy was readily demonstrated by applying a drop of fresh juice to a scratch. More recently, Ancona and Schumacher[3] have demonstrated similar results in patients allergic to shellfish. In 14 patients clinically sensitive to

shrimp, 3 different commercial extracts gave little or no reaction, but fresh or quick-frozen material gave positive reactions in scratch tests. Evidence has been offered that these reactions are specific. Preliminary studies indicate that a similar situation may apply to other groups of foods, as well as fruits and shellfish.

This superiority of a relatively primitive method of testing reflects our ignorance of the nature of antigenic materials in many foods. Presumably the fruits, berries, and shellfish contain antigens which are readily destroyed by enzymatic action or chemical manifestations, but are preserved in a stable form by quick freezing. Even antigens which remain active after cooking may be lost in the preparation of an extract. One patient who was clinically sensitive to peaches gave a marked reaction to a scratch test with the juice of home-canned peaches; the same juice after extraction and Seitz filtration gave no reaction on intracutaneous test.

Such results pose difficult problems, but also offer promise of substantial improvement in the results of food tests. To meet the demand for allergenic extracts on a wide variety of foods, the methods found suitable for extracting pollens have been applied, with such simple modifications as neutralization of excess acidity. The studies of Tuft and Blumstein and Ancona and Schumacher indicate that these methods are not applicable to many foods. Preparation of reliable allergenic extracts of these foods requires development of new methods, based on further knowledge of the properties of the antigens they contain. This field offers interesting possibilities for research. Pending a solution of this problem, Ancona and Schumacher have suggested the routine use of quick-frozen foods for scratch tests, and have presented a practical method of handling them. Further studies of the comparative results obtained by these two methods in patients clinically sensitive to all types of foods are needed to establish which extracts are efficient and which are not.

W. B. S.

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PRESENTATION OF HONORARY FELLOWSHIP IN THE AMERICAN ACADEMY OF ALLERGY TO PROFESSOR CESARE FRUGONI IN ROME, ITALY

THROUGH the good offices of Ambassador Dunn and the American Embassy, arrangements were made with the University of Rome for a presentation ceremony of the certificate of Honorary Fellowship in the American Academy of Allergy to Dr. Cesare Frugoni. The presentation took place in the Amphitheater of the Polyclinic at 11:00 A.M., Feb. 16, 1951. Il Rettore Giuseppe Cardinali of the University of Rome introduced Theodore L. Squier, retiring president of the Academy, who said, "It is my great privilege, on behalf of the American Academy of Allergy, to bring to one of Italy's great teachers greetings from his colleagues in the United States of America. In his long career as investigator, teacher, and recognized leader in internal medicine, Professor Cesare Frugoni has achieved eminence in his profession and world-wide renown. The great teacher and student, Sir William Osler, once said that honors were like bubbles, but that none were so brilliant as those which scintillated with the brilliant reflections of work done by the pupils of a great teacher. Although honors such as we today bring to Professor Frugoni are ephemeral, they represent our recognition of the undying fame which has been earned by him during his distinguished career as teacher, investigator, and physician. In the Middle Ages the science of medicine centered in the great medical schools of Italy. That tradition is continued today. Honors conferred on Dr. Frugoni serve also as an expression of the high regard physicians of the United States have for the many scientific contributions which have been made by our Italian colleagues.

Because of Professor Frugoni's outstanding position in Italy in internal medicine, because of his interest in allergy for over twenty-five years, during which he and his associates have carried out and published much important experimental and clinical research, and because of his long and distinguished career in teaching, both in internal medicine and allergy, the American Academy of Allergy, by unanimous action at its annual meeting in New York City, Feb. 6, 1951, has elected Professor Cesare Frugoni to Honorary Fellowship. I have been delegated further as official representative of Marquette University School of Medicine to convey greetings and good wishes from the dean and the faculty of Marquette University School of Medicine to Dr. Vittorio Puntoni, the president, and to the faculty of the School of Medicine and Surgery of the University of Rome, and to convey especially at this time their congratulations to Professor Frugoni.

"Professor Frugoni, on behalf of the American Academy of Allergy, it gives me great pleasure to present to you this certificate of Honorary Fellowship in the American Academy of Allergy."

Following translation into Italian, Dr. Vittorio Puntoni responded for the medical school. Professor Frugoni then made a most gracious speech of ac-

ceptance during which he announced that Dr. Squier and Dr. Francis Rackemann had been named honorary members of the Academy of Medicine of Rome. Dr. Squier then announced the election of Dr. Umberto Serafini, the first Italian active member of the Academy, referred to the objectives and ideals of the Academy, and, in welcoming him to membership, added that it

Dr. Theodore L. Squier presenting Fellowship Certificate of the American Academy of Allergy to Professor Frugoni in Rome, Italy. Left to right: Professor Cesare Frugoni, Il Rettore Giuseppe Cardinali, and Dr. Theodore L. Squier.

was hoped that through him and others there would be increasingly frequent and valuable contact with the important work in allergy and internal medicine in Italy.

On Feb. 19, 1951, Dr. Squier addressed the Academy of Medicine of Rome on "Progress in Allergy in the United States," and at that time received from Dr. Frugoni certificates of honorary membership in the Academy of Medicine.

Announcements

THE FIRST INTERNATIONAL CONGRESS OF ALLERGISTS

The First International Congress of Allergists will be held at Zurich, Switzerland, September 23-29, 1951, under the auspices of the International Association of Allergists. A preliminary program has been issued listing a large number of prominent speakers from Europe, Africa, and North and South America. The program may be obtained from Dr. Frederick W. Wittich, 401 LaSalle Medical Building, Minneapolis 2, Minn.

The Congress will be followed by a Symposium on the Influence of the Hypophysis and Adrenal Cortex on Biological Reactions arranged by the Swiss Academy of Medical Sciences on October 1 and 2, 1951.

Prof. Piero Sangiorgi, M.D., Secretary of the Italian Society of Allergy, will discuss "Geographical Distribution of Diseases Due to Allergies" at the First International Congress of Allergists to be held at Zurich, Switzerland, Sept. 23-29, 1951.

Prof. Sangiorgi asks all colleagues in every part of the world to send him information which may help him complete this work: that is, statistical data, regional clinic observations, publications, and so on. The names of the authors will be mentioned in the final report.

Address Prof. Piero Sangiorgi, M.D., Via Rivoli 2, Milan, Italy.

Communication

To: Fellows and Members of the Academy.

The Sub-Committee on Post Graduate Education is working on its plans for the current year and for 1952.

In preliminary discussions the following suggestions were made by members of the Sub-Committee:

(1) That future courses be held in conjunction with national meetings of our own organization or of others with related interests.

(2) That the number of additional courses, such as the one in Montreal, be limited to one or two a year—guided by regional needs and opportunities.

(3) That well-integrated symposia on selected—theoretical or clinical—phases of allergy be offered rather than courses on allergy as a whole. It was felt that courses which cover a wide area tend to become incongruous and rarely justify the effort of preparing or attending them. "Physiology of Respiration" and "Immuno-Chemistry of Allergy" were named as possible fundamental topics, "Allergy in Pediatrics" and "Allergy of the Skin" as clinical subjects.

(4) That a one-day "Seminar on Therapy" be presented to members of the Academy concurrently or immediately preceding its next meeting.

These are broad outlines. In order to become the basis of an effective program of post graduate instruction they must be modified (and translated into action) through critical appraisal and specific requests by individual members of the Academy.

We would appreciate your comment and your advice.

For the Sub-Committee,
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Book Reviews

Current Therapy 1951, edited by Howard F. Cohn, M.D., Philadelphia, 1951, W. B. Saunders Company, 699 pages.

This large volume offers the practitioner the opinions of more than 250 contributors on the treatment of a wide variety of diseases from schizophrenia to plantar warts. Since the writings of a large number of authors are assembled with a minimum of editing, the advice presented is exceedingly variable in quality. The discriminating reader, who knows the authors, will find much that is simple, practical, and new. The uninitiated may be confused by conflicting opinions of various authors. No references are given to guide those seeking further knowledge on controversial subjects. Some of the recommendations, such as aureomycin, 0.25 Gm. per kilogram of body weight per day, for the common cold, are so fantastic as to destroy confidence in the whole book.

The section on allergic diseases consists of 42 pages, contributed by 13 authors, and shows the uneven quality characteristic of the entire volume. This section contains little that is new and retains many old methods that are now widely discarded, such as strychnine sulfate for anaphylactic shock, Privine for hay fever, calcium, histamine injections, and histaminase for cold allergy, and unsaturated fatty acids for eczema. No mention is made of the use of ACTH and cortisone in asthma, the practical evaluation of which is probably the outstanding problem in the symptomatic therapy of allergic diseases at present. While the antihistamine drugs are discussed, one seeks in vain a useful comparison of the efficacy and toxicity of the various drugs available. Symptomatic treatment is stressed; the discussion of specific allergic study and treatment is too brief to be of practical value. The enthusiastic recommendation of Collergy tablets, a proprietary mixture of unstated ingredients, is out of place in a book seeking to be of scientific value. Surprisingly, urticaria and angioedema are not included among the allergic diseases, while sections are devoted to allergic arthritis and genitourinary allergy.

On the whole, this book contains much excellent material, but fails to maintain a uniform standard which is essential in a guide for the general practitioner. The allergist will find little of value in his own field.

The Management of the Patient With Severe Bronchial Asthma, by Maurice S. Segal, M.D., Springfield, Ill., 1950, Charles C Thomas, Publisher, 176 pp.

While recognizing the importance of allergic methods and psychotherapy in the handling of asthma, this little book essentially confines itself to an exposition of the drugs and other procedures of value in the symptomatic treatment of the asthmatic attack. In this field, directions and dosages are given in a concise, practical manner and procedures are described in adequate detail. The methods described are of proved value, but the book is in the unfortunate position of having been written before ACTH and cortisone were available, yet published after they were in common use, and found more effective than many of the procedures included.

An attempt is made to evaluate drugs on the basis of objective evidence and for this reason considerable space is devoted to their effects on asthma which has been induced with histamine and mecholyl. Some would question the applicability of data derived from the effects of drugs on such induced attacks to the treatment of clinical asthma.

In the treatment of asthma due to respiratory infection, antibiotic aerosols are stressed in preference to systemic administration of the same drugs, and the newer antibiotics, aureomycin, Chloromycetin and terramycin, all of which have proved useful, are not included.

Despite these reservations, this book is recommended as an interesting and valuable presentation of the subject.

Food Allergy, by H. J. Rinkel, M.D., T. G. Randolph, M.D., and M. Zeller, M. D., Springfield, Ill., 1950, Charles C Thomas, Publisher, 497 pages, $8.50.

This book presents in detail the revolutionary concept of food allergy already described by the authors in numerous papers. The familiar fixed type of food allergy, in which typical symptoms invariably follow the ingestion of the allergenic food, receives far less attention than cystic food allergy, in which periods of a few days of use or avoidance of the allergen produce fluctuations in the response from hyperacute sensitization to complete tolerance. The fluidity of these sensitizations is further emphasized by the statement that the use of a restricted diet of previously compatible foods may lead within one week to the acquisition of new sensitization. The complexity of the concept of cystic food allergy is indicated by the statement (repeated at least five times in various forms) that marked food allergy should be suspected from "the patient's assurance to the physician that he is not allergic to a certain food because he feels better every time he eats it. Such a food should be suspected as a cause of allergy until disproved by a specific ingestion test."

The diagnosis of this form of food allergy is based upon the occurrence of symptoms after ingestion of the suspected food during the stage of hyperacute sensitization induced by avoidance for four days. Leucopenia is considered corroborative evidence. Skin tests with foods are found to be only 20 to 40 per cent accurate.

The symtoms attributed to food allergy include in addition to the more familiar allergic manifestations, fatigue, marked pain, backache, rheumatoid arthritis, night sweats, urinary frequency, enuresis, nervousness, and insomnia.

The elimination diets recommended are extremely rigorous. Thus the corn-sensitive patient is advised to avoid not only the obvious sources, but also aspirin, bacon, commercial candy, harvard beets, carbonated beverages, cheese, grape juice, chewing gum, gin, cured hams, jellies, Rice Krispies, bleached wheat flour, and distilled vinegar.

It is apparent that this concept of food allergy bears little relation to that familiar to immunologists. No evidence is offered that this form of reaction has an immunologic basis, nor is any other type of mechanism suggested. Acceptance of such a concept must depend on carefully controlled studies in which both the patient and observer are at the time of study ignorant of the nature of the food being given and in which objective findings are given greater weight than subjective symptoms. It is questionable whether significant progress will be made by enthusiastic physicians applying to suggestible patients the methods described in the present volume.